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BEARING ON HEREDITY AND
VARIATION

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# GENETICS

**A Periodical Record of Investigations Bearing on Heredity and Variation**


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# THE FREQUENCY OF TRANSLOCATIONS PRODUCED BY X-RAYS IN DROSOPHILA

H. J. MULLER
*University of Texas, Austin, Texas*

AND

EDGAR ALTENBURG
*Rice Institute, Houston, Texas*

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By the term "translocation" is meant the breaking off of a piece of one chromosome and its attachment to another, non-homologous chromosome. An isolated case of a translocation was encountered in 1918 by BRIDGES (1923). Although involving too small a portion of chromatin to be noticed cytologically by the means available, it was thoroughly demonstrated by genetic means. This case stood alone, however, as a genetic oddity, unparalleled by similar cases that could be satisfactorily proved, until 1926. In that year STERN (1926, 1927) discovered a case in which a portion of the Y chromosome had become attached to the X; this was demonstrated both genetically and cytologically. Somewhat later in 1926, and in 1927, MULLER independently obtained genetic evidence that in his X-ray experiments numerous changes in gene alignment, of varied kinds, including translocations, had been produced (MULLER 1927a); some of these cases too were checked cytologically in 1927 (MULLER 1927b, 1928a, PAINTER and MULLER 1929). It then became of interest to discover with what frequency such translocations between non-homologous chromosomes might arise, as a result of exposure to short wave-length radiation, and of what different types these translocations might be.

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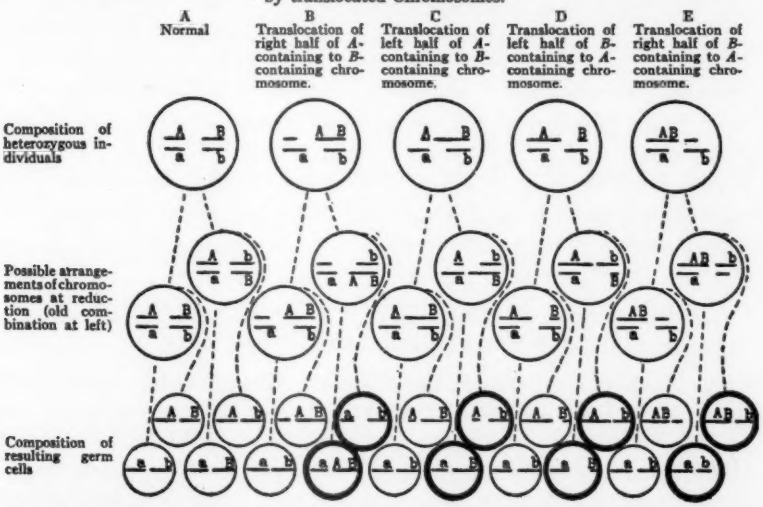
THE METHOD OF ATTACK

In order to investigate this question systematically, crosses were then started of irradiated males by non-radiated females which differed from the males in a number of "identifying genes" or "markers," located in different chromosomes, and some hundreds of the heterozygous offspring ($F_1$) were then backcrossed, in individual cultures, so that the distribution of the markers among the progeny of each of them could be determined. Ordinarily, the character differences dependent on different pairs of chromosomes would be distributed at random, according to Mendel's law of "independent segregation," but, if a translocation had occurred, the markers in the chromosomes involved would now appear as though linked.

By way of illustration, suppose that in one pair of chromosomes of the heterozygous $F_1$ which is to be tested there are the allelomorphic markers $A$ and $a$, and in another pair $B$ and $b$, so that the $F_1$ individual has, so far as these are concerned, a composition which can be represented by $\frac{A}{a}\ \frac{B}{b}$, (showing homologous chromosomes by parallel lines of different thickness, one below the other). Then, in the normal course of inheritance, the chromosomes might, of course, become arranged at reduction with equal likelihood either in the "old combination" $\frac{A}{a}\ \frac{B}{b}$, giving germ cells with $A\ B$ and with $a\ b$, respectively, or in the "new combination," $\frac{A}{a}\ \frac{b}{B}$, giving germ cells with $A\ b$ and with $a\ B$, respectively (see figure 1, diagram A). But if there had been a translocation in the sperm cell from which the $F_1$ had been derived, breaking the $A$-containing chromosome and tying the piece of it bearing $A$ to the $B$-containing chromosome, the $F_1$ composition would be represented by $\frac{A}{a}\ \frac{B}{b}$, and the chromosomes at reduction could have either the "old" arrangement $\frac{\ }{a}\ \frac{A}{b}\ \frac{B}{\ }$ or the "new" arrangement $\frac{\ }{a}\ \frac{A}{\ }\ \frac{b}{B}$. (If crossing over were possible still other arrangements could occur.) The first arrangement would yield the old combinations of markers $A\ B$ and $a\ b$, respectively, but the second arrangement would yield germ cells of composition $b$ and $a\ A\ B$, respectively. The former of these new-combination germ cells (namely $b$) and the progeny

resulting from it obviously have a deficiency of the genes in the right half of the originally *A*-containing chromosome, while the latter of the two new combinations carries an excess of genes of this same region. It is therefore likely that one or both of these new combinations will give abnormal and relatively inviable zygotes, or, in the case of a relatively large or potent translocation, that the new combinations will both be fully lethal.[1] Thus, so far as the non-crossover offspring are concerned, at any rate, only the old combinations of markers, *AB* and *ab*, may be found alive among the $F_2$, the combinations *Ab* and *aB* being absent, just as though *A* and *B* had been completely linked, or only the old combinations and one new-combination class will appear, or the new combinations may simply be less numerous and show phaenotypic abnormalities.

Figure 1.—Diagram showing nature of combinations produced by normal and by translocated Chromosomes.


A
Normal
B
Translocation of right half of A-containing to B-containing chromosome.
C
Translocation of left half of A-containing to B-containing chromosome.
D
Translocation of left half of B-containing to A-containing chromosome.
E
Translocation of right half of B-containing to A-containing chromosome.
Composition of heterozygous individuals
Possible arrangements of chromosomes at reduction (old combination at left)
Composition of resulting germ cells

(The germ cells in heavy outline contain combinations apt to be lethal to zygotes formed on backcrossing.)

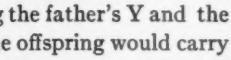
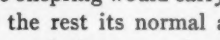
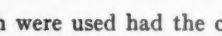
The same result would be just as apt to occur if the "left" end of the left hand chromosome had been attached to the right hand chromosome,

[1] It had been repeatedly insisted upon by MULLER in the earlier Drosophila work that a change from the normal proportions ("balance") of genes, caused by an excess or deficiency in the numbers of some genes in relation to the numbers of the others, would tend to produce phaenotypic abnormalities, including inviability. This conception is now fully proved by the present results even as regards disproportions involving the excess or deficiency of *parts* of chromosomes.

as follows: $\frac{A}{a}$ — $\frac{B}{b}$. For, even though $A$ and $B$ are not physically connected here, nevertheless, just as before, the two "new" non-crossover combinations, receiving one of these markers and not the other, will have a deficiency and an excess, respectively, of genes, and will hence tend not to be normal or viable. This result would also follow if either end of the $B$-containing chromosome should become attached to the $A$-containing chromosome, or, for that matter, if either the $a$- or the $b$-containing chromosome should become broken and have a fragment attached to the other. The failure of one or both new combination classes ($Ab$ or $aB$) to appear would therefore indicate a translocation between the non-homologous chromosomes containing the markers studied but would not by itself tell us which chromosome was broken and attached to the other nor which end of the broken chromosome had become attached. In some such cases it might even happen, theoretically, that both chromosomes ($A$-containing and $B$-containing) had become broken, mutually exchanging parts—a condition recently recognized by BLAKESLEE and BELLING in Datura under the term "segmental interchange," but these cases too would give results like the others, in the above tests. The objective of such tests, therefore, is to determine the total frequency of all kinds of translocations between the "marked" chromosomes which disturb the "genic proportions" sufficiently to cause the inviability, or the abnormality, of one or both of the new combinations.

## THE INITIAL RESULTS—A STARTLINGLY HIGH FREQUENCY OF TRANSLOCATION

In the first experiment (fall of 1927), the X-rayed males carried in one of their second chromosomes the dominant gene for curly wings ($C_y$), in the other second chromosome the dominant star eye ($S$), and in one of their third chromosomes the dominant dichaete ($D$), thus having the composition $\frac{X}{Y}$ $\frac{C_y}{S}$ $\frac{D}{\quad}$ (representing the sex chromosomes, the second pair and the third, in this order, but in the case of the autosomes, labelling only the mutant allelomorphs). They were given the treatment which has been designated as "t4" (MULLER 1928b) and immediately afterwards mated to non-rayed females having the normal allelomorphs of the above genes but carrying attached X-chromosomes with the genes for yellow body, and a supernumerary Y chromosome. The composition of the females might therefore be represented

as follows . The offspring from this cross which carried the father's X and the mother's Y would be males; those carrying the father's Y and the mother's attached X's would be females. Half the offspring would carry $C_y$, the rest would carry $S$. Half would carry $D$, and the rest its normal allelomorph, but only the $D$-carrying offspring were used, since a marker in the third chromosome was needed. If, now, we show the chromosomes derived from the rayed father by heavy lines, and again represent only mutant genes in the autosomes, the composition of half of the $F_1$ males used was  , and of the other half  . Males of both these types were then crossed with virgin normal females, one male to a culture, and the distribution of sex, curly or star, and dichaete, with regard to each other, among the progeny ("$F_2$") in each of the 90 fertile cultures was examined, to discover any cases in which any of the expected new combinations of the characters in question failed to appear, or appeared in very small numbers. $F_1$ females were also bred, one to a culture, by normal males. The females which were used had the composition  and ; thus these females also might reveal translocations, through counts of the distribution of sex, curly or star, and dichaete, with regard to each other.

It will be seen that the tests of the $F_1$ males were adapted to reveal translocations involving the rayed X chromosome and the second or third, while the tests of the females would reveal translocations involving the rayed Y chromosome and the second or third. In the case of the Y, however, only cases in which an autosome had been broken and attached to the Y would be discoverable by these methods, not the converse cases of breakage of the Y and its attachment to an autosome, since it is known that excess or deficiency of the Y chromosome or parts of it usually produce no change in viability or appearance.

It should be noted also, concerning the tests of the $F_1$ females, that $D$ does not serve as an absolute marker for the whole of the third chromosome as it does in the male, since crossing over can occur between it and other parts of this chromosome, in the female. Hence, in the tests of females, some cases of translocations involving the third chromosome may have gone unrecognized, and other cases might be recognizable only by the lessened frequencies of the "new combination" classes rather than by their

complete absence, inasmuch as crossing over might produce viable, genically balanced classes phenotypically simulating the inviable new chromosome-combinations that carry an excess or deficiency of genes. Similarly, crossing over might occur between star and a point of breakage or attachment in the second chromosome. Curly, however, formed a quite satisfactory marker for the whole of the second chromosome, where it was used, since it is associated with an unusual gene-arrangement that undergoes very little crossing over with a normal second chromosome. The close agreement of the results from the female tests with those from the male tests indicates, in general, that few of the translocations were missed in the former which would have been found in the latter.

The numbers of translocations found in this first experiment, and the chromosomes involved, are summarized in table 1, lines 1a and 1b, for the male and female tests separately. In all there were 21 translocations found among the 161 $F_1$ flies (representing as many X-rayed sperm cells of $P_1$ tested), or 13 percent. This was a startlingly high frequency, in view of the previous uniqueness of the phenomenon, and was comparable with the rate of detectable gene mutations occurring after X-raying. If the natural translocation rate is similarly high, compared with the natural rate of gene mutation, it must be of widespread, even though *relatively* infrequent, occurrence in nature and may be of importance in evolution.

Examining the sorts of translocations, it is seen that all the possible combinations of the chromosomes studied together were found: namely X-II, X-III, Y-II, Y-III, and II-III(where X-II means a translocation from either X to II or II to X; similarly for X-III, Y-II, etc.). The last class, II-III, was the most numerous, as might perhaps have been expected in consideration of the fact that it involved exclusively the two largest chromosomes. The relative frequencies of the different kinds of translocations will be considered in more detail later, when the results of all the experiments have been presented.

The counts from the individual cases of translocation are shown in table 2. Translocations involving II and III are evidenced by an absence or decided deficiency in numbers of the $C_y$ or $S$ non-$D$ and the non-$C_y$ or non-$S$ $D$ recombination classes. Translocations involving the Y or X and an autosome give results as though either $C_y$ or $S$ or $D$, as the case might be, had become sex-linked. Thus, if a piece of the second chromosome had become attached to the Y, then the $F_1$ males, on being bred to normal females, would transmit this piece to their sons, but not to their daughters. The curly (or star) daughters would therefore fail to

develop, since in them the second chromosome would lack the piece, and since the Y carrying the piece attached would also be absent. But in the curly male offspring this piece missing from the second chromosome would be attached to the Y chromosome and they could live. Hence, no curly daughters develop, but curly sons do. The non-curly sons, however, have the piece in triple amount, since one "dose" of it is attached to the Y, and two additional doses are present in their normal positions in the second chromosome pair, one of these normal chromosomes having been the untreated chromosome of the $F_1$ male, the other having come from the normal mother. On account of the genic disproportion thus produced, the non-curly males would fail to develop. The non-curly females, on the other hand, would not have the piece in triple amount (but simply in double amount) since they receive their father's X (not his Y, with the extra piece). Hence they live. In other words, in the case of a translocation of an autosome to the Y, all, or the great majority, of the flies of one sex show one of the autosomal traits involved, while the flies of the opposite sex show the allelomorph. Similar reasoning applies to an X-II translocation.

As expected, the counts from the tested $F_1$ females are not as clear-cut as from the males (on account of crossing over). From one of the females (case 15) a batch of progeny was derived in which a double translocation was evident, involving II, III and Y at the same time; here, since II and III gave the same results as though they were tied together, it was not possible to determine whether the translocation to the Y was from II or from III. (This accounts for the caption "sex and II or III" in table 1 at the head of the seventh column).

An additional kind of test was carried out in the case of the cultures derived from the tested $F_1$ females of this experiment. Here, owing to the method of inheritance imposed by the attached X's the $F_2$ males carried the Y chromosome that had been present in the rayed sperm of the original $P_1$ male, and that had been transmitted through the $F_1$ female of the composition that has been represented by $\overset{Y\rightharpoonup}{X_y X_y}\rangle$ If this Y chromosome had had a piece broken off by the X-rays, which had become lost or which had become translocated to a rayed autosome, then, although the $F_1$ female herself should be fertile (the Y seems to affect the female very little), nevertheless she should give rise to sons ($F_2$) inheriting the deficient Y, and, as STERN has shown, such males may be sterile. In the case of a complete loss of a fragment of the Y, all the sons ($F_2$) would be sterile; in the case of a translocation, all the sons receiving the non-radiated autosomes,

and therefore failing to receive the translocated fragment, would be sterile. All the males of the latter class (so far as they could be determined by the markers) were therefore tested, in mass culture, by normal females, in the case of each $F_1$-$F_2$ culture derived from a tested $F_1$ female, but no instances were found in which all males of the given class proved sterile. Nevertheless, direct tests of individual $F_1$ males from rayed $P_1$ fathers crossed by normal (not attached-X) females, showed considerable sterility, as MULLER had previously reported for his experiments. The above tests would hence indicate that most of this sterility in the $F_1$ males is not due to breakage of the Y chromosome.

### CONFIRMATION OF THE FINDINGS, AND DEMONSTRATION OF THEIR CONNECTION WITH THE TREATMENT

A second translocation experiment was then undertaken, involving a cross of X-rayed (t4) $S/C_v$ males by females having the combination of recessive genes in chromosome III termed "IIIpl" (roughoid hairy scarlet pink spineless ebony) The use of the multiple stock here, as well as in the following experiments, had no advantage over the $C_v$ (or $S$) $D$ stock in getting the translocation frequencies, but was used with a further purpose in mind The $F_1$ males in this second experiment carried the radiated Y Their composition may be represented as $\frac{Y}{X} \frac{S \text{ or } C_v}{IIIpl}$. These $F_1$ males were then used as $P_2$, being backcrossed, individually, to females carrying IIIpl or some part of IIIpl (in the combination $a_r$ $p_z$, $s_p$ "theca" referred to below) and the cultures were examined for the presence of recombinations among the progeny ($F_2$). As table 1, line 2 shows, 13 translocations of type II-III were found, and 3 involving the Y, in a count of 110. In this case, although the dosage factors were so far as possible the same as before, a new machine was used, which later tests have shown to give only a half to a third the effective output, when the controllable factors (time, distance, voltage, amperage, filter and surroundings) are sensibly the same as before. The counts from the translocations are shown in table 2, cases 21-36. In this instance, for some unexplained reason, the total translocation frequency is, if anything, even higher than before, but the disparity between the number of II-III's and those involving the sex chromosome is still evident.

In the second experiment 84 of the 110 $P_2$-$F_2$ cultures were kept track of in such a way that the recency of origin of the translocations found in them could be proved or disproved, that is, the fact that these transloca-

tions had not or had been present in the stock previous to the generation in which irradiation was carried out. This inquiry was undertaken as a substitute for the breeding of controls. The desired end was accomplished simply by recording the $P_2$-$F_2$ cultures in groups, any given group of cultures being "brother-cultures," in the sense that the $P_2$ males in all the cultures of this group were brothers, having been derived from the same treated $P_1$ male, and, in fact, carrying a derivative of the same second chromosome of that $P_1$ male (all cultures of the given group carrying $C_y$ or all carrying $S$). If, now, a given translocation, recognized from the $F_2$ count of one of these cultures, had really been present in the original $P_1$ male before treatment (having been received by him from one of his parents), it would have been transmitted by him to all of his progeny (the $F_1$ or "$P_2$") of the type used as $P_2$ in the group. The finding of a certain translocation (Y-II, Y-III, or II-III) in one culture of the group and not in another is therefore tantamount to the finding that this translocation did not arise in any generation antecedent to the $P_1$ male. Two fertile cultures in a group are enough to prove this point, and the conclusion would still be valid even if both of them contained translocations, if it could be shown that they did not contain the same translocation. The records of these cultures, by groups, are given in table 3.

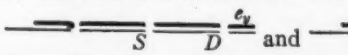
It will be seen that all of the 12 translocations found in these 84 cultures originated in what would be called the generation of the $P_1$ male. It is evident that this rate of origination must be far higher than what occurs in untreated material. For while little or no work has previously been done to determine the frequency of occurrence of translocations in control material, still many individual crosses involving genes in separate chromosomes have of course been carried out on Drosophila, and if translocations of natural origin occurred at all frequently, a fair number of natural cases should already have been found in the Drosophila investigations. If the 12 translocations here in question could have been regarded as an accumulated store, inherited from many previous generations, it might possibly have been contended that they might have been of "natural" origin, but when it is recognized that they must all have originated in one generation (the treated generation) it becomes obvious that the natural frequency of translocation cannot possibly be high enough to account for this, and that nearly all, or all of them, must have been due to treatment. This being the case it was considered unnecessary, for the purpose of the subsequent experiments, to run control tests in them or to keep track of the grouping of the $P_2$-$F_2$ cultures.

In a third experiment, radiated male flies having recessive genes in both the second and third chromosomes (in the second: arc, plexus, and speck; in the third: thread, stripe, sooty, rough, and claret, a combination termed "theca") were crossed to yellow females of the race with attached X's. The $F_1$ males, here carrying the radiated X, were tested, by backcrossing them to virgin females having the second and third chromosome recessives named. In the present experiment the new X-ray machine was used, and the factors of treatment would lead to a calculated dose of "t8" (about equivalent to the old "t3"). The numbers and kinds of translocations are given in table 1, line 3. It will be seen that the results are very similar to those of the first experiment. One of the translocations here too was a double one, involving II and III, and an attachment between the X and either II or III (which one being indeterminate from these counts). The individual counts from the cases of translocation are given in table 2, cases 37 to 55.

### EXPERIMENTS INVOLVING THE FOURTH CHROMOSOME

A series of experiments (4 to 7) was next undertaken which had as one of its objects the ascertainment of the frequency of detectable translocations involving the small fourth chromosome, as compared with those involving other chromosomes. The recessive mutant gene termed "eyeless" ($e_y$) versus its normal allelomorph was used as the marker for the fourth chromosome throughout these experiments, while the markers for chromosomes II and III remained *S* or $C_y$, and *D*, as in experiment 1. In experiments 4 to 6, however, all the dominants were brought into the $P_2$ flies from their non-radiated parents, while in experiment 7 the reverse was the case. The dosage was "t8" in experiments 4, 6, and 7, and "t12" in experiment 5, the new machine being used in each case.

The $P_1$ cross in experiments 4 to 6 was of irradiated "eyeless" males to females carrying Star (*S*) in one of their second chromosomes and Curly ($C_y$) in the other, and carrying *D* in one of their third chromosomes and in the other "$C_{III}x$" (a lethal suppressor of crossing over produced by a previous raying). The $F_1$ males chosen for breeding were those which had received *D*. These were therefore of the following composition:




again representing the irradiated chromosomes with heavy lines. These $F_1$ males were then used as $P_2$, by backcrossing them, one male to a culture, to virgin females having all recessive genes at the loci in question (namely, non-*S*, non-$C_y$, non-*D*, eyeless). The offspring ($F_2$) in each $P_2$-$F_2$ culture

were then examined in order to detect the absence of classes having recombinations of the markers in question (considered two at a time). It will be observed that the Y, II, III and IV were subject to study. The experience previously gained in the detection of translocations was now found to be sufficient to enable their detection to be effected without the making of counts, except in special cases.

In experiment 7 the irradiated $P_1$ flies were males of composition $\frac{(Y)}{(X)}$ $\frac{ST}{}$ $\frac{D}{}$ (only the mutant genes being represented, as usual.) Here *T* represents the gene for truncate wings, which was introduced with an ulterior object in view. So far as the determination of translocations is concerned, *T* may be disregarded here, since it has little expression in the heterozygous condition unless intensifiers are present in other loci; these were not present in this experiment. The $P_1$ females were homozygous for $e_v$, but otherwise normal. The $F_1$ flies which were used for breeding, as $P_2$, were males having the composition $\frac{ST}{}$ $\frac{D}{e_v}$ (again representing the treated chromosomes with heavy lines). As in experiments 4 to 6, they were backcrossed in individual cultures to recessive females (normal except for eyeless) and recombinations of all the markers, two at a time, were looked for.

The results of each of these experiments are summarized in table 1, lines 4 to 7. In all, 7 translocations involving the fourth chromosome were found, in a count of 420; all of these have been confirmed by later tests. It is practically certain, on theoretical grounds, that all of these cases were transfers from the large autosomes to the fourth. For not only is it probable that breakages of the fourth chromosomes would be very infrequent, as compared with breakages of the larger chromosomes, but, if they did occur, and were accompanied by an attachment of one of the fragments to a non-homologous chromosome, the recombinations carrying only such a small excess or deficiency of chromatin would almost surely be viable, in view of the known fact that individuals with an entire fourth chromosome extra or missing are also viable and not greatly different in appearance from the normal (the "triplo-IV's" and "haplo-IV's" of BRIDGES); hence such cases would probably not have been found by the methods used. Later analysis of the translocations in question has borne out this inference.

Curiously enough, two of the seven translocations to the fourth chromosome occurred in cases of double translocations; that is, in both these

cases two simultaneous translocations took place in the same treated sperm cell of the $P_1$ male. One of these cases was in experiment 5, where there was a simultaneous translocation between II and III, and to IV from either II or III, it being indeterminable which of the latter had its fragment attached to the fourth, inasmuch as all viable offspring that inherited the radiated II also inherited the radiated III. (This is why this translocation to the fourth had to be recorded as "II or III and IV.") The count of the $F_2$ cultures showed, instead of 16 classes in approximately equal numbers, only the following: $8 C_v D$ ♀, $5 C_v D$♂, $5 e_v$ ♀, $6 e_v$♂, 4 "normal" ♀. The "normals" were evidently triplo-IV individuals, caused by non-disjunction of the fourth chromosomes. Such non-disjunction might be expected, theoretically, owing to the large translocated autosomal fragment, through its affinities for its normally placed homologue, interfering with the synapsis and disjunction that should occur between the fourth chromosome to which it is attached and the normal fourth chromosome. The other case was in experiment 4, where the same $P_2$-$F_2$ culture showed both the II-IV translocation and also the Y-III translocation recorded in that experiment. The count in this case gave, instead of 16 classes: $12 S D$ ♀, $12 D e_v$♀, $11 S$ ♂, $5 e_v$♂. Later tests showed some non-disjunction of chromosome IV to occur in this case also.

Flies inheriting both the Y-III and II-IV translocations which arose in the case last described were then subjected to another irradiation, in order to produce a II-III translocation between these chromosomes, so that a stock giving no recombinations whatever of genes present in the father might be obtained. This end was readily accomplished, one of the desired translocations being found in a batch of 25 $P_2$-$F_2$ cultures from a second irradiation with a t11 dose. Males of the resultant Y-II-III-IV translocation stock, then, always transmit this entire set of chromosomes to their fertile male offspring (though some sterile non-disjunctional males also occur), and these offspring show a similar genetic behavior, as do all fertile descendants in the direct male line. The female offspring, on the other hand, except where non-disjunctional, must always receive the entire homologous set of chromosomes that their father contained, just as though the chromosomes of each haploid set had been joined into a single chain.

In experiments 4 to 7 care was taken not to overlook cases in which one recombination class was absent while the contrary recombination class was present in the $F_2$ cultures. Three such cases involving II and III were found (1 each in experiments 4, 5, and 6), and they have been included

among the II-III translocations in table 1. In the case in experiment 4 the class ($C_y$) containing the non-radiated second and the radiated third chromosome was viable; in the cases in experiments 5 and 6, on the other hand, it was the opposite recombination which was the viable one. There is reason, based on analysis of other cases, to believe that when only one of two contrary recombination classes derived from Drosophila translocations is viable, that class is usually the "hyperploid," the one carrying the overdose of certain genes, while the lethal recombination is the class having the deficiency of genes, that is, the "hypoploid." If this is true, then the first of the translocations just referred to involved a transfer from II to III, while the other two involved transfers from III to II.

## COMPARATIVE FREQUENCIES OF DIFFERENT TRANSLOCATIONS

We may now take count of the comparative frequencies of the translocations involving different chromosome combinations. In experiments 4 to 7 there were 7 translocations from II or III to IV and 8 or 9 translocations from II or III to Y (transfers in the opposite direction being excluded in the case of both these kinds of translocations, as previously explained). It therefore seems as though fragments of the long autosomes were about as likely to become attached to the small fourth as to the Y chromosome. When, however, we examine the results of the other experiments involving the Y, we find a somewhat higher frequency than in experiments 4-7 of the Y-II and Y-III translocations, when the latter are compared with the frequency of II-III translocations as a standard of reference. It may be, therefore, that the observed frequency of Y-II and Y-III translocations in experiments 4-7 was the result of a chance fluctuation that caused it to be somewhat below the mean value. Since the II-IV and III-IV frequencies would not necessarily fluctuate in the same direction it is possible that the translocations to the Y are really somewhat more numerous than to chromosome IV; this difference in frequency, if it exists at all, cannot be very great, however, and certainly is not proportionate to the difference which exists between the physical sizes of these chromosomes, as cytologically observed.

In order to compare the frequencies of translocations involving the Y and the X, respectively, we may again find each frequency in terms of its relation to the frequency of II-III translocations found in the same experiment, using the latter as our standard of reference. From table 1, on the line marked "Total for X, II, III," we find that there were 8 translocations of types X-II plus X-III, as compared with 18 of types II-III, that is, a ratio

of 1:2.2. On the line below this we find that there were 17 (or 18) translocations of types Y-II plus Y-III, as compared with 67 of type II-III, a ratio of 1:3.7. Thus these figures indicate a higher (apparently nearly twice as high) incidence of translocations involving the X than of translocations involving the Y. The absolute number (8) of X translocations found is, however, too small for us to be certain of the "significance" of such a difference in frequency.

Such a difference as above indicated would be expected if the translocations of types "X-II" and "X-III" found include translocations in both directions (from II to X and also from X to II, etc.). For, as previously stated, the translocations found involving Y were always in the same direction—from the autosome to the Y, those from the Y to the autosome having remained undetected. However, there is evidence that a somewhat similar limitation applies also in the case of the X, when the present methods of detection are employed. For, as we shall see later, breeding tests have indicated that a translocation is usually accompanied by the appearance of a recessive lethal in the chromosome which underwent breakage. Thus a broken X, received by an $F_1$ male, would usually cause the death of the latter, even though both fragments were present. It was the $F_1$ males, (derived from crosses of radiated $P_1$ males by attached-X females) which were used (experiments 1a and 3) in tests of translocations involving the X chromosome. Hence classes "X-II" and "X-III" probably represent almost exclusively translocations in the direction "II to X" and "III to X." The three translocations involving the X which appeared in experiment 1a were analyzed genetically, and all of them proved to be in this direction. Translocations in the opposite direction can occur, for in other experiments of MULLER, in which females containing irradiated X's were tested, translocations in the direction, from the X to the autosome, have been found; these in most, but not all, cases have proved lethal in the male.

If, then, we consider our present classes "X-II" and "X-III" as being in only one direction, the difference in frequency between these and the Y translocations appears more pronounced, and the difference in frequency between the translocations involving the X and those involving IV cannot be due to "fluctuations of sampling" (8 translocations to X in an experiment yielding only 18 of type II-III, and 7 translocations to IV in an experiment yielding 48 of type II-III). The results will, however, be less equivocal in an experiment in which all types can be studied at once, since there is some indication that the frequencies of different types of translocations, relative to one another, may also be subject to variation due to determinate causes.

Regardless of our conclusions concerning the frequencies of the translocations to X, to Y, and to IV, respectively, relatively to each other, there is no room for doubt that the frequency of each of them is very significantly lower than the frequency of type II-III. Since class "II-III" is in reality composed of the translocations from II to III plus those from III to II, it is legitimate to compare its frequency with that of the sum of X-II and X-III or the sum of Y-II and Y-III, or the sum of II-IV and III-IV, inasmuch as any single one of these other classes comprises translocations exclusively, or nearly exclusively, in one direction, namely, from the larger to the smaller chromosome, being in this respect unlike II-III. As table 1 shows, there were, in experiments yielding 18 of class II-III, 8 of classes X-II plus X-III; there were, in experiments yielding 67 of class II-III, 17 or 18 of classes Y-II plus Y-III; and there were, in experiments yielding 48 of class II-III, 7 of classes II-IV plus III-IV. These give ratios of 1:0.45, 1:0.27, and 1:0.15, respectively. In brief, then, the figures 1:0.45:0.27:0.15 represent the relative likelihoods, as indicated by our present data, of a fragment from a long autosome becoming attached to the other long autosome, to the X, to the Y, or to the fourth chromosome, respectively.

The cause of this variation in the likelihood of attachment to the different chromosomes is still obscure. Light may be shed upon it through more detailed analysis of the structures of the individual translocations; this is a project now well under way. If, for example, a considerable proportion of the translocations were mutual, involving breakage of and transfer from and to both chromosomes concerned at once (BLAKESLEE's "segmental interchange"), it would follow that (so far as these mutual translocations were concerned) their frequencies might be roughly proportional to the products of the lengths of the two chromosomes taking part (supposing that the chance of breakage in a chromosome is roughly proportional to its length). As yet, however, the evidence seems against most of the translocations in Drosophila being mutual. Again, we might account to some extent for the apparent relation between the length of a chromosome and the likelihood of its serving for the attachment of a fragment from another if the attachment point could be anywhere along its length, rather than always terminal. This would not necessarily require the fragment to become attached to the side of a chromosome—a process that might lead to difficulties in its growth and longitudinal division. It might also come about if the "recipient" chromosomes sometimes underwent a breakage simultaneous with that of the "donor" chromosome, accompanied by reunion of the pieces in a different arrangement from that of before ("inversion" or "in-

sertion"), one or both of the breakage points thus serving for the attachment of the fragment from the donor.

Neither mutual translocation nor any kind of mutual breakage, nor side attachment, can well be the rule, however, else we should expect a much greater disparity between the different classes of translocations than actually exists. For the figures 1.0:0.45:0.27:0.15 by no means express the relative lengths of the chromosomes respectively serving as recipients; these are more nearly given by the figures 1.0:0.67:0.67:0.01. The products of the lengths show still greater disparities. In various cases already analyzed, moreover, it has been proved that the recipient chromosome underwent no breakage or rearrangement, and that the attachment was terminal, though mutual translocations have also been found.

Still other problems would be raised if it should be found that the relative frequencies of the different kinds of translocations differ in different experiments. For example, a comparison of the figures for experiments 5 and 6, in table 1, suggests that in the former there were significantly fewer translocations to Y and IV, in proportion to the II-III translocations, than there were in the latter. If such differences were to be substantiated, we could no longer use the II-III class as a fixed standard of reference, and our relative figures would have meaning only when considered in connection with certain other determining conditions in the experiment, of a nature at present unknown.

### THE EFFECT OF TRANSLOCATIONS WHEN HOMOZYGOUS

Reference has been made in the foregoing to the finding that the majority of the translocations were lethal when homozygous. Experiments 3 to 6, inclusive, were purposely framed in such a way that no lethals (for example, $C_v$, $S$ or $D$, which are all lethal when homozygous) would be in the radiated chromosomes to begin with; these therefore would not prevent the obtainment of the translocated chromosomes in homozygous condition. Various specimens of translocations, of different kinds, involving X, Y, II, III, and IV, have now been sampled, in tests constructed for the purpose of getting them homozygous. Relatively few could be obtained homozygous, and those that could have usually shown sterility and different kinds of morphological abnormalities, when homozygous, just as though they contained a gene-mutation or as though, like the hyperploid and hypoploid types, they were genically disproportioned. The detailed evidence on these points must be reserved for another paper.

Three possible explanations present themselves for this at first sight surprising situation.

(1.) There has been a simultaneous gene mutation (these are usually

lethals) at some other locus in the chromosome, at a distance from the locus of breakage or attachment. Such a coincidence, occurring in the majority of cases, seems highly unlikely. However, there is some as yet inconclusive experimental evidence that one genetic change tends to be associated with another elsewhere in the same cell, so we can not abruptly dismiss this possibility as yet.

(2.) The alteration in intermolecular surroundings of the genes directly adjacent to the points of breakage and reattachment, in other words the alteration in intergenic contiguities, has in itself brought about a change in the quantity or quality of the physico-chemical action of these genes upon the protoplasm, so as to make them, in effect, somewhat different genes, as though gene-mutations had taken place in the genes on either side of the breakage and attachment points. Plausibility is lent to such an assumption through STURTEVANT'S finding that two genes for bar eye adjacent to one another in the same chromosome seem to have an amount of effect on the development of the eye different from that of two otherwise identical genes for bar eye that lie in separate, homologous chromosomes. Fundamental problems of genetics are involved here which merit active prosecution.

At present we have at least one ray of light on the point here at issue. If this assumption is true, then the genes next to the attachment point on the recipient chromosome should become changed in their action just as much as the genes next to the breakage point on the donor chromosome. Nevertheless, in the cases where there was the possibility of distinguishing such an effect—the cases of translocation *to* the X, 3 or 4 of which have been studied in some detail, and in which the male serves to reveal any changes in genes of the X—no lethal action or visible abnormalities could be detected. It is true that these may only count as one case in point, since the attachment was in each instance to the right hand end of the X and it might be assumed that this was for some reason a locus particularly insensitive to the sort of change in question. However, till further evidence is forthcoming,* these cases must be taken as opposed to the present interpretation.

(3.) There is finally the possibility that in the act of breakage a chromosome usually becomes injured, so that one or more of the genes next to the breakage point either become destroyed, or are caused to mutate in some way. (Not that they are made functionally different by their changed relationships, as in possibility 2, in which case a reestablishment of the original alignment would automatically restore the original genotype

* More light on the question will be obtained when a study is made of the effect of attaching extra segments to the end of X chromosomes which have, through previous gene rearrangement, acquired new terminal points.

exactly, but that they have been permanently altered by some "injurious" process which accompanied the breakage, but which was not inseparable from the latter.) By a process of elimination, this possibility seems for the time being the most probable. It agrees in general with BRIDGES' interpretation of why his original translocation was not viable when homozygous—namely that the attached piece did not represent quite all of the originally detached piece, a small portion next to the breakage point having become lost in the process of breakage or transfer. It is not, however, necessary to suppose that the small portion was lost; it may merely have been caused to mutate. A breakage, if it could occur in the middle of a gene, instead of between genes, would be bound to cause such a loss or at least mutation; this possibility merges into that of alternative 2. If the genetic material be regarded as a continuum, instead of as segmentally arranged in units, the genes, there would be no distinction between an intra-genic and an inter-genic break, and all breaks would partake of the nature of gene-mutations, as postulated in 2. Reasons have, however, been given (MULLER 1926–1929) for regarding the genetic material as segmental.

The above being the apparent possibilities, they must be kept in mind so that as the evidence concerning translocations accumulates it may be considered constructively, in its bearing on these questions, and also so that new methods of experimental attack on them may if possible be evolved. But no matter which of the possible causes, if any, should turn out to be correct, one apparent effect of the phenomenon in question is so obtrusive that it cannot be ignored here. That is, since most translocations are found to be lethal when homozygous, and the remainder seem commonly to be sterile and abnormal, it would at first sight seem highly unlikely that translocations could play a role in the evolutionary processes occurring in nature. For the establishment of the ordinary translocation in homozygous condition, in a group of individuals, would be tantamount to exterminating the group.

The situation is, however, not so impossible as it might seem at first sight. If the translocations are accompanied by gene mutations, or by genetic changes similar in their effect to gene mutations, it is only to be expected that the majority of them all would be lethal, and that, of the remainder, which were not lethal, the majority would be antagonistic to survival and reproduction. For, as is well known, that is true of the gene mutations themselves, and it is just what is to be expected of any random changes occurring in a complex organization. Nevertheless, evolution must have proceeded through gene mutations, that is, through the very rare gene mutations which happened not to be detrimental, and which there-

fore could withstand the test of natural selection. The numerous lethals, which perished, were thus only a necessary concomitant of the same process which produced the relatively few mutations of a non-detrimental character. The same may therefore be true also of translocations and other changes in gene alignment. In fact, the somatic or physiological changes commonly accompanying translocations may from this point of view be considered as an aid to their becoming established in a species, for then there might be a few adaptive translocations which would have the aid of natural selection in spreading through a group of the species. On the other hand, if the translocations never had any somatic expression their spread would occur only under extraordinary accidental circumstances, as through a series of successive decimations of a population, in each of which only a few chance-chosen individuals survived; undoubtedly, however, such events happen more than once in most groups, in the course of thousands of generations.

### THE FREQUENCY OF TRANSLOCATIONS AS COMPARED WITH THAT OF GENE MUTATIONS.

Although the survival of translocations, provided they occur, thus presents no insuperable obstacles, the question of their frequency of occurrence remains to be considered. If their frequency is far below that of ordinary gene mutations we should expect far fewer translocations than gene mutations to become incorporated in a stock in the course of its evolution during a given period. Evidence derived from a comparison of the cytology, and of the genetic maps, of related species of Drosophila, indicate this to be almost certainly true [see the work of METZ (1914, 1916) and of STURTEVANT 1921, STURTEVANT and PLUNKET 1926], though *some* changes in gene alignment, both inter- and intra-chromosomal, must undoubtedly have occurred since the origination of the genus. Direct experimental evidence on the present question is lacking, since no systematic searches for translocations in control material, similar to the mutation frequency studies of the authors, have been made. If, however, many of the mutations in somatic characters which have been observed in untreated material had been accompaniments of translocations or of other gene rearrangements, considerable positive evidence of this should, it seems, already have been adduced in all the locus determinations which have been made of the Drosophila mutations. And such evidence has not so far appeared.

From the present studies, on the other hand, we may obtain some direct evidence regarding the frequency of translocations induced by X-rays, as compared with gene mutations similarly induced. In all the data here

presented, together, there was a total of 117 translocations found, each of which involved at least one breakage of one of the long autosomes, and its attachment to some non-homologous chromosome or other in the same cell. There would probably have been about 8 more such translocations found, if chromosome IV had been studied in all the experiments, thus making a more complete figure of 125. These were distributed among a total of 883 tested $F_1$-$F_2$ cultures, from $P_1$ males given an average X-ray dosage of "t9" (in terms of the newer machine). The frequency of translocations from both of the long autosomes was therefore 125/883 or 14 percent. Thus we must conclude that a given one of the long autosomes, subjected in the sperm cells to treatments of about t9 strength, becomes broken and translocated to some non-homologous chromosome—X, Y, IV, or the other long autosome—in approximately 7 percent of the treated sperm cells. (Correction is not made here for mutuals or for possible differences in frequency to X and to Y.)

This figure of 7 percent for a "t9" dose may then be compared with the figure 12 percent, which represents the number of detectable "mutations"—mainly lethals—found by MULLER in 1926 and 1927 in the X-chromosome of sperm cells given the equivalent of a "t12" dose ("t4" on the old machine) (MULLER 1928b), or with the figure 8 percent, the percentage of X-chromosome lethals found by HARRIS this year (HARRIS 1929), following a "t8" (new machine) treatment of the sperm. It must, however, be remembered that the long autosomes are about one and a half times as large as the X, both cytologically and genetically. In a given portion of a long autosome about the size of the X, then, we should find only about $2/3 \times 7$ percent, or 4.7 percent of breakages accompanied by translocations, with a t9 dose, or, if the effect is proportional to dosage, about 0.5 percent with a t1 dose. This, then, is to be compared with 1.0[3] percent of visible plus lethal "mutations" in the X-chromosome per each "t1" of dosage. The rate of translocations detectable by the present methods in the space of a "unit" in one of the long autosomes is therefore just about half the rate of "mutations" detectable by the methods used for these, in about the same space on the X-chromosome. These "mutations," however, themselves included a considerable proportion of chromosome abnormalities. It is likely, moreover, that at least half of the translocations which occurred escaped detection. This follows from the fact that any particular fragmentation breaks the chromosome into a fiber-bearing fragment (the longer fragment, in the case of the long centrally attached autosomes), and a fragment without fiber attachment. If now the fiber-bearing fragment of a chromosome became attached to another

[3] Oliver's recent work shows 1.25 percent to be the more nearly correct figure here.

chromosome, an abnormal chromosome, having two points of fiber attachment, would become formed, while the other fragment, lacking any fiber, would fail to be transported in cell division, and an inviable offspring would probably ensue. The real rate of occurrence of translocations may therefore be just as high as, or higher than, that of gene mutations, after X-ray treatment has been applied to the sperm cells.

This conclusion is rather surprising, if, as now seems likely from the work of HANSON (1929a) and of OLIVER (1930), the genetic effectiveness of X-rays remains proportional to dosage, and if in addition natural mutations and translocations should be mainly due to "natural X-rays." For in that case we should expect as many natural translocations to occur as natural detectable "point mutations." We can be virtually sure that this is not the case. Owing to the property which translocations have of being usually accompanied by lethal or other detectable phaenotypic effects, a large number of them should have been found in the work of discovery and analysis of apparent gene mutations in Drosophila, if the translocations were really comparable in numbers to the gene mutations, even though translocations as such had not been specifically looked for. The absence of cases in the earlier work thus indicates a real scarcity of them, as compared with the frequency of ordinary gene mutations. There can, however, be no doubt about the high frequency of the translocations artificially induced in the present experiments, and the fact that this frequency must have been comparable with that of the gene mutations here. How then shall we reconcile these two conflicting series of findings?

It must be remembered that the natural cases dealt with represent a sum total of the mutations that have occurred at all different stages of the life cycle, and in either sex, indiscriminately. All the induced translocations and gene mutations considered, on the other hand, occurred at one restricted stage—namely, in the mature spermatozoa. There is some evidence, from work of HANSON (1929b) and of HARRIS (1929), that the germ plasm at this stage is peculiar in its sensitivity to radiation. At least, considerably more of both gene mutations and chromosome abnormalities occur in offspring from cells radiated in the spermatozoan stage than in those from other rayed cells; conceivably, however, this may be due to a higher selective death rate of the mutant cells in stages other than that of the spermatozoa. Whether the gene-mutation frequency or the chromosome-abnormality frequency is raised more has not as yet been determined. However, work of MULLER (as yet unpublished) on the relative

TABLE 1

| NUMBER OF EXPERIMENT | X-RAY MACHINE USED | X-RAY DOSAGE | SEX OF $F_1$ TESTED | TOTAL FERTILE CULTURES | NUMBERS OF TRANSLOCATIONS INVOLVING: SEX AND II | SEX AND II or III | SEX AND III | II AND III | SEX AND IV | II AND IV | II OR III AND IV | III AND IV | CHROMOSOMES STUDIED |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | old | t4 a | ♂ | 90 | 2 | | 1 | 6 | | | | | X II III |
| | | t4 b | ♀ | 71 | 3 | 1 | 2 | 6 | | | | | Y II III |
| 2 | new | t4 | ♂ | 110 | | | 3 | 13 | | | | | Y II III |
| 3 | " | t8 | ♂ | 192 | 2 | 1 | 2 | 12 | | | | | X II III |
| 4 | " | t8 | ♂ | 44 | | | 1 | 4 | | 1 | | | Y II III IV |
| 5 | " | t12 | ♂ | 172 | (1?) | | 1 | 23 | | | 1 | | Y II III IV |
| 6 | " | t8 | ♂ | 159 | 3 | | 1 | 15 | | 3 | | 2 | Y II III IV |
| 7 | " | t8 | ♂ | 45 | 2 | | | 6 | | | | | Y II III IV |
| Total for X, II, III (1a; 3) | | | | 282 | 4 | 1 | 3 | 18 | | | | | X II III |
| Total for Y, II, III (1b, 2, 4, 5, 6, 7) | | | | 601 | 8(+1?) | 1 | 8 | 67 | | | | | Y II III |
| Total for II, III, IV (4, 5, 6, 7) | | | | 420 | | | | 48 | | 4 | 1 | 2 | II III IV |
| Entire Total | | | | 883 | 12(+1?) | 2 | 11 | 85 | 0 | 4 | 1 | 2 | (X or Y) II III (IV). |

Among the cultures in experiment 5 were 2 in which all the offspring ($F_2$) contained the nonradiated second chromosome, bearing Curly. There were 28 offspring in each of these cultures. Evidently a dominant lethal had been induced in chromosome II; the viability of the $P_2$ male containing it may have been due to its not having contained the lethal in all tissues of its body (owing to the fractional effect often shown by mutants in the first generation after radiation of the sperm) or it may have been due to an incompleteness of action of the lethal (the latter then being really a "semi-lethal"). One clear case of a dominant semilethal induced in chromosome II, that produced a disarrangement of the bristles, was also encountered in experiment 5, and one dominant semilethal, producing various thoracic abnormalities, was found associated with a II-III translocation yielding a count of 21 *$C_y$ D* and 2 non-*$C_y$* non-*D* (variants).

A "delta"-like vein abnormality, located in the radiated third chromosome, was found in one of the cultures of experiment 6 not involving a translocation.

TABLE 2

TABLE 2

*Progeny counts in $F_2$ cultures involving translocations and other abnormalities.*

| EXPERIMENT NUMBER | CASE NUMBER | SEX OF $F_1$ TESTED | SEX CHROMOSOMES TESTED | MARKERS USED IN | | NUMBERS OF FLIES RECEIVING RADIATED CHROMOSOMES AS INDICATED BY MARKERS ($S_x$=*radiated* X OR Y) | | | | | | | | TYPE OF ABNORMALITY | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | II | III | $S_x$ II III | II III | $S_x$ | — | $S_x$ II | II | $S_x$ III | III | | |
| 1. | 1–6 | ♂ | X | *S* or $C_y$ | *D* | √* | √ | √ | √ | 0 | 0 | 0 | 0 | II–III | |
| " | 7 | " | " | " | " | √ | 0 | 0 | √ | √ | 0 | 0 | √ | X–II | |
| " | 8 | " | " | $C_y$ | " | 36 | 0 | 0 | 32 | 28 | 0 | 0 | 19 | " | small piece or much non-disjunction |
| " | 9 | " | " | " | " | 11 | 6 | 1 | 10 | 1 | 15 | 8 | 1 | X–III | |
| " | 10 | ♀ | Y | " | " | 14 | 10 | 24 | 11 | 0 | 0 | 0 | 0 | II–III | |
| " | 11 | " | " | " | " | 23 | 10 | 14 | 25 | 0 | 0 | 0 | 0 | " | |
| " | 12 | " | " | " | " | 17 | 15 | 12 | 16 | 0 | 0 | 0 | 0 | " | |
| " | 13 | " | " | " | " | 14 | 6 | 23 | 11 | 0 | 0 | 0 | 0 | " | |
| " | 14 | " | " | " | " | 12 | 23 | 33 | 5 | 0 | 1 | 2 | 3 | " | some crossing over between *D* and translocation |
| " | 15 | " | " | *S* | " | 8 | 1 | 0 | 8 | 1 | 0 | 0 | 0 | Y–II–III | double translocation; some non-disjunction and crossing over |
| " | 16 | " | " | " | " | 8 | 0 | 0 | 14 | 17 | 0 | 0 | 19 | Y–II | |
| " | 17 | " | " | $C_y$ | " | 11 | 1 | 1 | 9 | 15 | 1 | 1 | 8 | " | some non-disjunction |
| " | 18 | " | " | " | " | 9 | 1 | 2 | 5 | 4 | 0 | 0 | 7 | " | " " " |
| " | 19 | " | " | " | " | 9 | 0 | 0 | 9 | 0 | 7 | 2 | 0 | Y–III | |
| " | 20 | " | " | " | " | 15 | 3 | 8 | 15 | 9 | 16 | 10 | 1 | " | much crossing over |
| 2. | 21 | ♂ | " | *S* | III pl‡ | 22 | 17 | 10 | 18 | 0 | 0 | 0 | 0 | II–III | |
| " | 22 | " | " | " | " | 25 | 14 | 1 | 10 | 0 | 0 | 0 | 0 | " | |
| " | 23 | " | " | " | " | 17 | 26 | 8 | 6 | 0 | 0 | 0 | 0 | " | |
| " | 24 | " | " | $C_y$ | " | 18 | 16 | 8 | 9 | 0 | 0 | 0 | 0 | " | |
| " | 25 | " | " | *S* | " | 15 | 11 | 11 | 10 | 0 | 0 | 0 | 0 | " | confirmed in further tests |
| " | 26 | " | " | " | " | 8 | 13 | 12 | 13 | 6 | 7 | 0 | 0 | " | hyperdiploid classes viable |
| " | 27 | " | " | " | " | 3 | 3 | 4 | 0 | 0 | 0 | 0 | 0 | " | confirmed in further tests |
| " | 28 | " | " | " | " | 14 | 11 | 15 | 14 | 0 | 0 | 0 | 0 | " | " |
| " | 29 | " | " | " | " | 9 | 17 | 11 | 11 | 0 | 0 | 0 | 0 | " | " |
| " | 30 | " | " | " | " | 8 | 13 | 6 | 14 | 0 | 0 | 0 | 0 | " | " |
| " | 31 | " | " | " | " | 22 | 14 | 24 | 13 | 0 | 0 | 0 | 0 | " | " |
| " | 32 | " | " | $C_y$ | " | 20 | 16 | 27 | 18 | 0 | 0 | 0 | 0 | " | " |
| " | 33 | " | " | " | " | 10 | 10 | 16 | 20 | 0 | 0 | 0 | 0 | " | " |
| " | 34 | " | " | *S* | " | 14 | 0 | 0 | 15 | 0 | 15 | 20 | 0 | Y–III | " |

TABLE 2. (*Continued*)

*Progeny counts in $F_1$ cultures involving translocations and other abnormalities.*

| EXPERIMENT NUMBER | CASE NUMBER | SEX OF $P_1$ TESTED | SEX CHROMOSOMES TESTED | MARKERS USED IN | | NUMBERS OF FLIES RECEIVING RADIATED CHROMOSOMES AS INDICATED BY MARKERS ($S_x$ = *radiated* X OR Y) | | | | | | | | TYPE OF ABNORMALITY | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | II | III | $S_x$ II III | II III | $S_x$ | — | $S_x$ II | II | $S_x$ III | III | | |
| 2 | 35 | ♂* | Y | *S* | IIIpl | 1 | 0 | 0 | 1 | 0 | 3 | 5 | 0 | Y–III | confirmed in further tests |
| " | 36 | " | " | $C_y$ | " | 2 | 0 | 0 | 2 | 0 | 7 | 6 | 0 | " | " |
| 3. | 37 | " | X | "aps | theca†"‡ | 10 | 10 | 37 | 53 | 0 | 0 | 0 | 0 | II–III | multiple-mutants have low viability in this experiment |
| " | 38 | " | " | " | " | 2 | 8 | 22 | 17 | 0 | 0 | 0 | 0 | " | |
| " | 39 | " | " | " | " | 3 | 11 | 13 | 25 | 0 | 0 | 0 | 0 | " | " |
| " | 40 | " | " | " | " | 17 | 15 | 47 | 60 | 0 | 0 | 0 | 0 | " | " |
| " | 41 | " | " | " | " | 28 | 26 | 21 | 37 | 0 | 0 | 0 | 0 | " | " |
| " | 42 | " | " | " | " | 27 | 29 | 33 | 49 | 0 | 0 | 0 | 0 | " | " |
| " | 43 | " | " | " | " | 16 | 12 | 28 | 30 | 0 | 0 | 0 | 0 | " | " |
| " | 44 | " | " | " | " | 9 | 12 | 16 | 27 | 0 | 0 | 0 | 0 | " | " |
| " | 45 | " | " | " | " | 6 | 7 | 37 | 47 | 0 | 0 | 0 | 0 | " | " |
| " | 46 | " | " | " | " | 9 | 10 | 29 | 27 | 0 | 0 | 0 | 0 | " | " |
| " | 47 | " | " | " | " | 10 | 16 | 25 | 32 | 0 | 0 | 0 | 0 | " | " |
| " | 48 | " | " | " | " | 10 | 0 | 0 | 47 | 0 | 0 | 0 | 0 | X-II-III | double translocation |
| " | 49 | " | " | " | " | 9 | 1 | 1 | 34 | 14 | 1 | 0 | 18 | X–II | some non-disjunction |
| " | 50 | " | " | " | " | 1 | 0 | 0 | 7 | 13 | 0 | 0 | 17 | " | |
| " | 51 | " | " | " | " | 5 | 0 | 0 | 49 | 0 | 11 | 21 | 0 | X–III | |
| " | 52 | " | " | " | " | 6 | 0 | 1 | 17 | 0 | 23 | 12 | 0 | " | |
| " | 53 | " | " | " | " | 0 | 0 | 10 | 14 | 0 | 0 | 14 | 17 | dominant lethal in II | |
| " | 54 | " | " | " | " | 1 | 0 | 24 | 32 | 0 | 0 | 0 | 0 | dominant lethal and II–III | or contamination; not counted as translocation |
| " | 55 | " | " | " | " | 0 | 0 | 72 | 67 | 0 | 0 | 0 | 0 | | |

* The symbol √ indicates that some flies of class indicated were present in each of the cultures in question, but number was not recorded.

† "aps theca" = $a_7\ p_2\ s_9\ i_h\ s_7\ e^2\ r_0\ c_a$.

‡ The markers "III pl," "aps" and "theca" were present in the non-radiated $P_1$ eggs; the markers $C_y$, *S*, and *D*, when used, were present in the radiated $P_1$ sperm.

effects of radiation on immature female germ cells and on spermatozoa indicates that the chromosome-abnormality frequency is increased much more in the spermatozoa as compared with the oögonia, than is the gene-mutation frequency. It is therefore possible that the high rate of translocations, as compared with gene mutations, observed in the experiments reported in the present paper, is a phenomenon especially pronounced in the spermatozoa.

Although the above consideration might partially account for the discrepancy in question, it is probably by no means sufficient to explain all of it, for undoubted displacements of chromosome sections have been found following irradiation of other cells than spermatozoa. It is therefore likely that there is a real difference between X-rays and the "natural" cause or causes of mutations, in regard to their relative effectiveness in altering the genes and in causing breakages and reattachments of parts of chromosomes. This conclusion is strengthened by the evidence, obtained by MULLER and MOTT-SMITH since the present paper was written, that natural radioactivity is not the cause of most mutations in untreated Drosophila.

## SUMMARY

1 a. Methods are described for detecting the occurrence of translocations in Drosophila, based upon the principle that the effect of genic disproportion (excess or deficiency) involving even sections of chromosomes will often be sufficient to cause the inviability or abnormal appearance of one or both recombination classes of zygotes.

b. The results obtained by the use of these methods amply demonstrate the validity of the theoretical conceptions upon which they are based.

2 a. The frequency of translocations following heavy irradiation by X-rays has been found to be surprisingly high. In all, in the experiments herein described, 117 translocations, all or nearly all of them involving at least one breakage of one of the long autosomes and the attachment of a fragment of it to a non-homologous chromosome, have been found in a total of 883 fertile $P_2$-$F_2$ cultures derived from irradiated $P_1$ males.

b. The percentage of such breaks and reattachments of a given long autosome is thus at least 7 percent. Unit for unit of the chromosome, and dosage for dosage, this is just about half the rate of occurrence of detectable mutations of all kinds, as found in the X chromosome.

c. When allowance is made for the fact that translocations involving the attachment of the fiber-bearing instead of the fiberless fragment are not included in the above figure of 7 percent and that translocations too small to

EXPLANATION OF TABLE 2

This table can be explained by considering specifically experiment No. 1, cases 1–6. The sex of the $F_1$ fly tested was male, as indicated in column 3. The sex chromosome tested, as indicated in column 4, was the X, because the radiated father was mated to an attached X female; he therefore transmitted his X (rather than his Y) to his $F_1$ male offspring. The markers used in chromosomes II and III, as indicated in columns 5 and 6, were *S* (star) or $C_y$ (curly), for chromosome II, and *D* (dichaete) for chromosome III. These genes are dominants. The $F_1$ male receives two of them, *S* (or $C_y$) and *D* from his radiated male parent. He receives the normal allelomorphs, *s* (or $c_y$) and *d*, from his normal, unradiated mother. In the $F_1$ males, sperm cells of four classes (so far as these markers are concerned) are formed by Mendelian segregation, namely, *SD*, *Sd*, *sD*, and *sd* (or $C_y$ in place of *S*), and these contain, respectively, the radiated II and III chromosomes, the radiated II and unradiated III, the unradiated II and radiated III, and the unradiated II and unradiated III. When these $F_1$ males are mated to normal females, offspring ($F_2$) that recieve the radiated II and III chromosomes will appear *SD* (that is, star dichaete). Those that receive the radiated II but the normal III will appear *Sd* (star, not-dichaete), etc. Column 7 in the table is headed by the caption $S_x$ II and III, and experiment 1, cases 1–6, are checked for these chromosomes; that is, there appeared some flies in the $F_2$ that were star and dichaete, and that therefore received II and III from their radiated grandparent. Being male, they also received a sex chromosome ($S_x$) from him. The next column is headed II and III, and below it is a check. This means that some flies appeared that received II and III from their radiated grandparent, but that, being female, they did not receive his radiated sex chromosome, as indicated by the absence of the symbol $S_x$ in this column. The ninth column is headed "$S_x$" and again is checked below. This means that some flies appeared which received $S_x$, but neither II nor III from their radiated grandparent. The dash in the following (tenth) column, with a check below, indicates that some $F_2$ flies appeared that had not received $S_x$ or the II or III from their radiated grandparent. In the eleventh column, headed $S_x$ II, there is an O. This indicates that no flies appeared in the $F_2$ that received the sex and the II, but not the III, from the radiated grandparent. In the twelfth column, headed simply by II, there is also an O, showing that flies which failed to receive the radiated sex chromosome, but received the radiated II, and not the radiated III, likewise were missing. Columns 11 and 12, taken together, thus show that, regardless of the sex chromosome, flies with II but without III from their radiated parent could not live. In a similar manner, columns 13 and 14 indicate that flies that received only the III chromosome of their radiated grandparent, and not his second also, could not live. In other words, in experiment 1, cases 1–6, flies that received II without III, or III without II, could not live and did not make their appearance in the $F_2$. This fact indicates a translocation from II to III or from III to II, and is accordingly recorded as such in column 15, under the heading "Type of abnormality." In the remaining experiments, after case 7, (namely, experiments 1, 2 and 3, cases 8 to 55), the various viable classes of $F_2$ offspring were counted, instead of being simply "checked" for the appearance of a fairly large number of offspring belonging to these classes. The counts are indicated in columns 7 to 14.

result in readily detectable effects of the genic disproportion also are not included, whereas all kinds of chromosome abnormalities as well as "point mutations" involving lethal or other phaenotypic effects are included in the count of lethals in the X, it is seen that the frequency of occurrence of induced translocations is probably at least as high as, or higher than, that of induced gene mutations of a detectable kind.

d. This finding requires reconcilement with the apparent rarity of "natural" translocations, as compared with "natural" gene mutations.

The explanation may partly lie in an especial liability to chromatin displacements on the part of spermatozoa, which were the type of cells chosen for treatment. But it is also probable that the natural causes of mutations differ from X-rays in that they cause a much higher frequency of gene mutations, as compared with chromatin rearrangements, than do X-rays.

3. In the second experiment reported it was demonstrated that the translocations were not previously present in the stock, but arose in the cells of the treated generation.

4 a. A fragment broken off of either of the long autosomes (chromosomes II or III) can become attached to any of the other chromosomes present, namely, the other long autosome, the X, the Y, or the small fourth chromosome.

b. The relative frequencies of occurrence of the above various kinds of attachment, as found in the present experiments, formed the following ratios, respectively: (to II or III) 1: (to X) 0.45: (to Y) 0.22: (to IV) 0.15.

c. Among these differing frequencies, we may at least attach significance to the greater value of the first frequency (representing attachments to the other long autosome) as compared with each of the other frequencies (representing attachments to the smaller chromosomes).

d. The reason for this greater tendency for attachment to a long autosome is still undetermined, though possible explanations are suggested. Thus it may be that a breakage occuring in one chromosome favors the attachment to it of a fragment from another chromosome, and that breakage is more likely to occur in a longer chromosome. Breakage of a recipient chromosome is, however, not *necessary* for the occurrence of attachment.

5 a. Breakages of and translocations from the X chromosome have been found to occur in other work of the authors, although the technique of the present experiments largely prevented their detection here.

b. Breakages and translocations of part of the Y to other chromosomes seem, according to some tests herein reported, to be less frequent than those of the long autosomes (none having been found in a rather limited series of tests).

6 a. It has been found that a large proportion of the induced translocations produce lethal effects on zygotes which receive them in a homozygous condition. Those which are not lethal commonly produce sterility and other somatic manifestations, when homozygous.

b. Three possible reasons for such effects have been discussed—simultaneous mutation at a separated locus, possible effect on gene functioning of a

change in its gene associates, and mutation or loss of genes at or adjoining the points of breakage (or attachment). At present the last hypothesis seems the most probable.

c. These lethal and other deleterious effects of most translocations, when homozygous, do not rule out translocations as factors in evolutionary change any more than the lethal or deleterious character of the vast majority of detectable gene mutations rules out gene mutations as the main building blocks of evolution. It is not to be expected that the majority of any changes occurring at random willl have survival value.

TABLE 3

*Results from individual $P_1$ males in portion of second experiment.*

| A<br>DESIGNATION OF $P_1$ MALE | B<br>MARKER CARRIED BY $P_1$ MALES | C<br>NUMBER OF FERTILE $F_2$–$F_3$ CULTURES FROM "A", CARRYING MARKER INDICATED IN "B". | D<br>NUMBER OF CULTURES IN "C" CARRYING TRANSLOCATIONS | E<br>TYPE OF TRANSLOCATION | F<br>CASE NUMBER OF TRANSLOCATION, AS REFERRED TO IN TABLE 2 |
|---|---|---|---|---|---|
| 1 | $S$ | 5 | | | |
| 2 | $S$ | 3 | 1 | II–III | 25 |
| 3 | $S$ | 2 | | | |
| 4 | $S$ | 12 | 1 | II–III | 26 |
| 5 | $S$ | 7 | 1 | II–III | 27 |
| 8 | $S$ | 2 | | | |
| 9 | $S$ | 3 | | | |
| 10 | $S$ | 6 | 1 | II–III | 28 |
| 11 | $S$ | 5 | 2 | II–III | 31 |
| | | | | Y–III | 34 |
| 12 | $S$ | 3 | | | |
| 13 | $S$ | 2 | | | |
| 15 | $S$ | 1 | | | |
| 16 | $S$ | 6 | 1 | II–III | 29 |
| 17 | $S$ | 6 | 1 | II–III | 30 |
| | $C_v$ | 3 | 1 | II–III | 32 |
| 18 | $C_v$ | 3 | 1 | II–III | 33 |
| | $S$ | 3 | | | |
| 19 | $S$ | 2 | | | |
| | $C_v$ | 6* | | | |
| 20 | $S$ | 2 | 1 | Y–III | 35 |
| | $C_v$ | 2 | 1 | Y–III | 36† |
| Total | | 84 | 12 | 9II–III; 3Y–III | |

* Dominant minute bristles, completely linked with $C_v$, appeared in 1 culture.

† Different translocation from number 35.

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# STUDIES ON INHERITANCE IN PIGEONS. VII.
# INHERITANCE OF RED AND BLACK COLOR PATTERNS IN PIGEONS[1]

W. R. HORLACHER

*The Agricultural and Mechanical College of Texas, College Station, Texas*

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## INTRODUCTION

The hereditary relations existing among the various self colors of pigeons (*Columba livia*) have been worked out by COLE and his co-workers (COLE 1911, 1912, 1914, COLE and KELLEY 1919) and by CHRISTIE and WRIEDT (1923). There are numerous color patterns in pigeons, almost each variety and sub-variety presenting a pattern somewhat different from that of any other. Some of these patterns, particularly those due to the dominant gene *A*, were studied in some of the papers mentioned above by COLE and his students. The epistatic series of black, black blue-tail, check, sooty, blue barred and blue barless was worked out by JONES (1922) and studied later by BOL (1926). Various color patterns involving mixtures of red and black have been reported on by METZELAAR (1926, 1928). STAPLES-BROWNE (1908) described "kitiness" as early as 1906, but he used

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the term to designate the "buffy edgings" which sometimes occur on black feathers.

The experiments reported on in this paper, which were begun in 1925, deal chiefly with the inheritance of two color patterns in pigeons in which there are mixtures of red and black pigment.

Facilities were adequate at all times so that each mating was made in an individual mating cage. Thus the matings were controlled and there can be no question as to the parentage of the birds produced as there might have been had the matings been made with several pairs in a large fly.

Some of the matings considered were made at the UNIVERSITY OF WISCONSIN. Several of the original pigeons used in the matings made in Texas were obtained from the UNIVERSITY OF WISCONSIN and hence had Wisconsin numbers. The Texas number and the Wisconsin numbers can be readily distinguished by the fact that a Texas number is preceded by a T, thus: T27A, whereas no letter precedes a Wisconsin number. All the progeny from a single mating are given the same number followed by different letters. Thus the progeny from mating No. T92 are given the numbers T92A, T92B, T92C, etc.

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## DESCRIPTION OF PATTERNS

The inheritance of each of two different patterns showing red and black was studied. The first of these patterns shows an intimate mixture of red and black pigment in all or in a part of the feathers. The best way to describe it is that there appears to be a sprinkling of both colors over the entire feather. This color pattern is found in the Brander, the Almond Tumbler, and the Bronze Tippler. LYELL (1887) describes the Brander as follows: "The Brander is a Copenhagen Tumbler, of a black ground color, but strongly glossed with reddish bronze. A good bird should appear all over of the same color as that on the breast of the bronzed Archangel pigeon, except that the points of the primary and secondary flights, as well as a bar across the end of the tail, should be black. The Brander is smooth-headed and legged, and is, doubtless, the breed referred to by NEUMEISTER as the Fire Pigeon."

Pigeon fanciers have always been troubled with the occurrence of blacks and blues which show a bronze luster, or a red or fiery glow over and through the ground color. This condition is called kitiness, probably on

account of the similarity in appearance of kitey pigeons with the European kite (*Milvus ictinus*). Kitiness appears most commonly in the primary wing feathers. It may, however, be found over the entire bird in which case a pigeon similar to one of the breeds mentioned above is produced.

COLE (1914) in studying the genetic relationship of the self colors black and red observed that sometimes the black pigeons resulting from such crosses had more or less kitiness.

It has not been possible to photograph this type of pigeon in such a manner as to show the pattern. The flecks of the two colors are very small. Each barb of the feather may have both colors. In fact, both red and black pigment granules sometimes occur in the same barbule and sometimes even in the same cell within a barbule. Figures 1 and 2 are

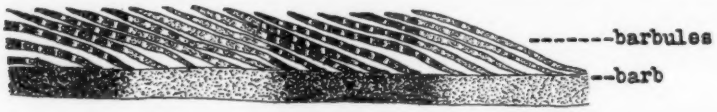



FIGURE 1.—Barbules on one side of a barb, showing black with kitiness. (X360. The red, light stippling, and black, heavy stippling, pigments are mixed at and near the border lines between the red and the black areas. This is not apparent because of the use of an uncolored plate. Camera lucida drawing from feathers of T58B, black with kitiness.)

FIGURE 2.—Hooked barbule containing both pigments. (X660. Heavy stippling indicates black pigment; light stippling indicates red pigment. Camera lucida drawing from feathers of T58B, black with kitiness.)

camera lucida drawings of a portion of a feather from a pigeon of this type showing an intimate mixture of the two colors of pigments.

As will be shown below, the pattern in the different breeds of which it is characteristic is due to the same gene as that which produces kitiness in other breeds. Hence, these are all grouped together and will be referred to in this paper under the term kitiness.

The other red and black pattern studied is a very definite pattern. It is found in the Archangel pigeon. Certain parts of the body show a definite red, or, as the fancy calls it, bronze, color. These parts are the head, neck, breast, thighs and underparts, and it extends to the elongated feathers beneath the tail. The remaining portions of the bird have black plumage. These parts are the wings, back, rump and tail. The line of demarcation between the black and the bronze areas should be very distinct. Figure 3

is a non-tinted picture of an Archangel showing the parts which are black and those which are bronze.

It is a fault that is fairly prevalent in Archangels that more or less black in flecks or small areas appears on the surface of the bronze. The underfluff in this region is black. The black sometimes appears in considerable quantities on the head and upper part of the neck, thus destroying the

FIGURE 3.—The Archangel. The lighter areas are black; the darker areas bronze.

uniform bronze appearance. Sometimes in dark bronze Archangels so much black appears in these regions that they look black rather than bronze.

According to GOODALL the best Archangels have no bronze mixed in with the black. Each Archangel used or produced in these experiments, however, had some bronze flecking in the wings. Some Archangels show only a very slight bronze flecking. In these the flecking is in the inner vanes of the primaries and is not visible when the wings are folded. Others show more flecking in various amounts. In some there is sufficient bronze in

the wings to give them a decided reddish cast. FULTON(1895) states that there should be some bronze in the inner vanes of the primaries.

There are two types of Archangels, known as the dark bronze and the light bronze. These differ with regard to the shade of the bronze. The bronze on the dark bronze Archangel has a rich copper color. That on the light bronze is golden yellow in color.

Another characteristic of the Archangel, the inheritance of which was studied in the experiments reported in this paper, is the crest. This has nothing to do with the color pattern. The crest consists of a tuft of feathers at the back of the head compactly drawn together and culminating in a needle-like point. It should point upwards and not backwards. It varies somewhat in different pigeons with regard to its compactness and shape. An idea of the crest can be gained from figure 3.

The Archangel is known in most countries as the bullfinch pigeon. The dark bronze is known in Germany as the Kupfergimpel and the light bronze as the Goldgimpel.

## EXPERIMENTAL RESULTS

### *Studies concerning kitiness*

#### Relationship to black

Black and kitey birds were mated in matings numbers T1, T2, T3, T5, and T6. The results of these matings are shown in table 1.

TABLE 1

*$P_1$ cross Black×Kitey.*

| MATING NUMBER | KITEY | BLACK |
|---|---|---|
| T1 | 3 | 0 |
| T5 | 6 | 0 |
| Total—T1 and T5 | 9 | 0 |
| T2 | 4 | 2 |
| T3 | 3 | 3 |
| T6 | 1 | 2 |
| Total—T2, T3 and T6 | 8 | 7 |
| All matings | 17 | 7 |

The matings are divided into two groups, those that produced only kitey offspring and those that produced both black and kitey offspring. The kitey pigeons used in these matings differed in their genetic make-up. Those which produced only kitey progeny were homozygous for gene *K* which produces kitiness, whereas those which produced both black and kitey progeny were heterozygous for this gene *K*.

Various matings were made between $F_1$'s from the above matings. Also backcrosses were made to both parental types. In mating T92 a black $F_1$, T3C, was backcrossed to a black, 2014C. Six young were raised from this mating, of which five were black and one was dun. No kitiness segregated out.

Two black $F_1$'s, T3A and T3F, were mated in mating No. T47. Nine young were raised from this mating, of which six were black and three were red. No kitiness segregated out. The production of the recessive red progeny shows that both T3A and T3F were heterozygous for gene *E*, the gene for the extension of black pigment.

Kitey $F_1$'s were backcrossed to blacks in matings T59, T62, and T63, with the results shown in table 2.

TABLE 2

*$P_1$ Kitey×Black. $F_1$ Kitey backcrossed to black.*

| MATING NUMBERS | KITEY | BLACK | RED |
|---|---|---|---|
| T59 | 1 | 3 | 0 |
| T62 | 2 | 1 | 1 |
| T63 | 3 | 3 | 0 |
| Observed | 6 | 7 | |
| *Calculated 1:1* | *6.5* | *6.5* | |

Deviation $0.5 \pm 1.2141$

Assuming that kitiness is a simple monohybrid dominant to black, a ratio of one kitey to one black is expected. It will be shown later that it cannot be determined without the proper breeding test whether or not a recessive red pigeon carries the gene *K*. Hence, in classifying the progeny from the matings given in table 2 for gene *K* the red bird is not classed in either group. The results of this backcross are very close to a 1:1 ratio.

Several matings were made between black and kitey $F_1$'s, with the results shown in table 3.

TABLE 3

*$P_1$ Kitey×Black. $F_1$ Kitey×$F_1$ Black.*

| MATING NUMBER | KITEY | BLACK |
|---|---|---|
| T48 | 1 | 5 |
| T60 | 2 | 2 |
| T61 | 2 | 2 |
| T90 | 2 | 7 |
| Observed | 7 | 16 |
| *Calculated 1:1* | *11.5* | *11.5* |

Deviation $4.5 \pm 1.6188$.

This cross is the same as a backcross and a 1:1 ratio is expected from it. There was a marked deviation from this ratio, a deviation which is almost three times its probable error.

All the matings of kitey pigeons heterozygous for gene *K* with blacks have been given above, but in three separate tables. In order that they may all be gotten together and the results summarized, they are presented again in table 4.

TABLE 4

*All matings of Kk pigeons with blacks.*

| MATING NUMBER | KITEY | BLACK | RED |
|---|---|---|---|
| T2 | 4 | 2 | 0 |
| T3 | 3 | 3 | 0 |
| T6 | 1 | 2 | 0 |
| T59 | 1 | 3 | 0 |
| T62 | 2 | 1 | 1 |
| T63 | 3 | 3 | 0 |
| T48 | 1 | 5 | 0 |
| T60 | 2 | 2 | 0 |
| T61 | 2 | 2 | 0 |
| T90 | 2 | 7 | 0 |
| Observed | 21 | 30 | 1 |
| *Calculated* | *25.5* | *25.5* | |

Deviation $4.5 \pm 2.4084$

The agreement of the results from all these matings with a 1:1 ratio is fairly close, the deviation being less than twice its probable error.

Three matings were made of kitey $F_1$'s *inter se* with results as shown in table 5.

TABLE 5

*$P_1$ Kitey×Black. $F_1$ Kitey×$F_1$ Kitey.*

| MATING NUMBER | KITEY | BLACK | RED |
|---|---|---|---|
| T46 | 8 | 5 | 3 |
| T50 | 7 | 0 | 0 |
| T57 | 1 | 0 | 0 |
| Observed | 16 | 5 | 3 |
| *Calculated 3:1* | *15.75* | *5.25* | |

Deviation $0.25 \pm 1.3381$

In this case two heterozygous, *Kk*, pigeons were mated together. On a monohybrid basis a 3:1 ratio was expected. The agreement is very close.

In order to determine whether or not the kitiness which occurs occasionally in black and blue pigeons is inherited in the same manner as the kiti-

ness studied above, the breeding records kept by COLE when he was studying the relationship of black and red were searched and a study made of the inheritance of kitiness in these pigeons. The skins, wings, or feathers of a great many of these birds have been saved. These were examined whenever possible as a check on the written description. In several instances it was doubtful from the description whether or not the bird in question was kitey. If no parts had been saved it was not possible to check up on the written description. In case the kitiness should prove to be due to the gene *K*, it should be inherited in exactly the same manner as it has been demonstrated that the kitiness studied above is inherited. Tables 6, 7 and 8 are taken from Doctor COLE's records.

In the matings given in table 6 each parent was black with kitiness.

TABLE 6

*Black, with kitiness×Black, with kitiness.*

| MALE PARENT | FEMALE PARENT | KITEY | NOT KITEY |
|---|---|---|---|
| 194B | 559A | 4 | 0 |
| 972A | 559A | 2 | 2 |
| | Totals | 6 | 2 |

In presenting the data which were taken from Doctor COLE's records, the band numbers of the two parents are given rather than the mating number. The reason for this is that in his earlier records he gave a separate mating number to each clutch of eggs. Hence, each pair of pigeons was given as many mating numbers as the number of clutches of eggs that it produced.

If this kitiness is due to a single dominant gene the expectation from matings such as those given in table 8 is either that all the offspring will be kitey or that they will be produced in the ratio of 3 kitey to 1 non-kitey. The agreement with these expectations in table 6 is close.

Table 7 gives the results from matings in which a black pigeon with kitiness was mated with a black pigeon without kitiness.

TABLE 7

*Black, with kitiness×Black, no kitiness.*

| KITEY PARENT | NON-KITEY PARENT | KITEY | NOT-KITEY |
|---|---|---|---|
| 714, female | 28B, male | 7 | 7 |
| 702A, male | 844A, female | 0 | 2 |
| | Totals | 7 | 9 |

Evidently both of the kitey parents shown in table 9 were heterozygous. These matings, then, are backcrosses of the heterozygote on the recessive type from which a 1:1 ratio is expected. The ratio secured, 7:9, deviated only one from the expected, 8:8.

Matings between black pigeons with no kitiness are given in table 8.

TABLE 8

*Black, no kitiness×Black, no kitiness.*

| MALE PARENT | FEMALE PARENT | KITEY | NOT-KITEY |
|---|---|---|---|
| 45B | 56A | 1 | 18 |
| 8A | 35A | 0 | 11 |
| 8A | 7A | 0 | 3 |
| 8A | 411A | 0 | 5 |
| 157A | 411A | 0 | 8 |
| 969A | 411A | 0 | 2 |
| 999A (Blue) | 7A | 0 | 10 |
| 888A (dun) | 976B | 0 | 11 |
| 966A (dun) | 884A | 0 | 1 |
| 1093A | 1093B (dun) | 0 | 5 |
| 1484C (blue) | 1093B (dun) | 0 | 3 |
| 46A | 46B | 1 | 4 |
| 6A | 7A | 0 | 15 |
| 493B | 454B | 0 | 14 |
| 448A | 432B | 0 | 7 |
| 862A | 862B | 1 | 0 |
| | Totals | 3 | 117 |

If black with no kitiness is the recessive condition, all of the progeny from the matings given in table 8 should be black without kitiness. The numbers here are comparatively large. One hundred and seventeen of the progeny from these matings were black with no kitiness. But three were kitey. These are exceptions to the hypothesis which has been given to explain the inheritance of kitiness.

It is said by some pigeon fanciers that once a pair of pigeons are mated they will remain mated for life, and furthermore, that they may be placed in a large fly with other pigeons but will never mate with them. Working with this idea in mind, Doctor COLE made all his earlier matings in large flies. From time to time results appeared which were not in accordance with the expectation. It seemed probable that this was due to the fact that the matings were not strictly controlled. A pigeon is, perhaps, not as true to its mate as some fanciers believe it to be. If there is an inequality of the sexes in a fly, which may result from the death of one member of

a pair, or for some other reason, the odd bird or birds are very likely to mate with some of the birds which are otherwise mated. Also, a hen occasionally lays in the wrong nest, resulting in the egg being credited to the wrong parents. As a result of the general unsatisfactoriness of this system, it was abandoned and the present system of using individual mating pens was adopted. Regarding this change, COLE and KELLEY (1919) say: " .... these early results were obtained at a time when the matings were not controlled as closely as they have been in the later years of the work. Until 1911 the mated pairs were not isolated but were kept together in large pens, though special care was taken to prevent crossmating and to detect it if it occurred. Beginning in 1912, the use of separate pens for pedigreed matings was begun, and this practise was extended so that there were very few non-isolated pairs in 1914 and all matings have been strictly controlled since that time."

It seems plausible, therefore, to explain the occurrence of the three kitey birds noted in table 8 as being due to unavoidable errors in mating, resulting from the system then in use. The preponderance of evidence from Doctor COLE'S records is that two non-kitey black pigeons when mated together do not produce kitey offspring.

The results of the matings given above prove that kitiness is dominant to self black in pigeons and that this pattern is produced by the action of a single gene which has been given the symbol *K*.

### Relationship to recessive red

It has been shown by COLE (1914) that black in pigeons is a simple Mendelian dominant to red, the difference being due to the gene *E* which is responsible for the extension of black pigment over the entire bird. In the recessive condition, *ee*, the black is not extended and the red color has an opportunity to express itself. Knowing this fact, and also that kitiness is dominant to black, it was to be expected that kitiness would prove to be dominant to red. Several matings of red with kitey birds were made with the results shown in table 9.

The possibility that self red, self black, and kitiness form a series of multiple allelomorphs similar to that in guinea pigs (IBSEN 1919) has been suggested. These results disprove this theory. If these did constitute such a series, the order, on the basis of the previously known relationship of self black and self red and of the relationship established above between self black and kitiness would have to be kitiness at the top, or the dominant member of the series, self red at the bottom, or the recessive member of the series, and self black in the middle dominant to self red and recessive

to kitiness. A kitey bird which was heterozygous could then carry either the gene for self black or the gene for self red in a recessive condition, but not both. A recessive red bird could carry neither the gene for self black nor the gene for kitiness because either one, if present, would be expressed in the phenotype of the bird and it would not be red. A mating of a kitey bird which was heterozygous with a self red bird could then produce either some self red or some self black offspring, but not both, the type produced depending on which of the two lower members of the series was in the heterozygous bird. Three of the matings given in table 9, T49, T82, and T86, did not produce both self black and self red offspring, thus indicating that the theory of multiple allelomorphs does not offer the correct interpretation of the situation. Further evidence that this theory does not offer the correct interpretation is given below when it is shown that a self red pigeon can carry the gene which produces kitiness.

TABLE 9

*Red×Kitey.*

| MATING NUMBER | KITEY | BLACK | RED |
|---|---|---|---|
| T49 | 0 | 2 | 3 |
| T73 | 0 | 0 | 2 |
| T82 | 1 | 1 | 4 |
| T83 | 1 | 0 | 3 |
| T86 | 2 | 2 | 2 |
| T87 | 3 | 1 | 0 |
| T89 | 0 | 0 | 4 |
| T116 | 2 | 0 | 0 |
| Observed | 9 | 6 | 18 |
| *Calculated 1:1:2* | *8.25* | *8.25* | *16.5* |

$P=0.6781$

The results given in table 9 can be interpreted on the following basis, which seems to be the correct one. Black is a simple dominant to red, the difference being due to the gene *E*. Kitiness is a simple dominant of self black, the differences being due to the gene *K*. The gene *K* can produce kitiness only when *E* is present. It is a pattern gene affecting black. A self red bird can carry the gene *K* but it will not be expressed because there is no black on which it can be expressed. Matings of kitey birds homozygous for both *E* and *K* with double recessive self red birds should produce only kitey offspring, thus: $EEKK \times eekk \rightarrow EeKk$. If, however, the kitey birds used are heterozygous for both genes, and previous results from the mating of kitey with self black showed that the birds used in these experi-

ments were heterozygous in some instances at least, the three types would be expected in the ratio of 1 red and black: 1 black: 2 red, thus:

*EeKk* × *eekk*
↓
1 *EeKk* kitey
1 *Eekk* black
1 *eeKk* red
1 *eekk* red

This interpretation fits the observed results very closely as shown in table 9.

According to the above theory one-half of the self red birds resulting from the matings in table 9 should be heterozygous for the gene *K*, *Kk*, and the other half should be recessive, *kk*. The test for the gene *K* in a self red bird is to mate it with a self black. Any kitey progeny resulting from such a mating is due to the gene *E* from the black parent and the gene *K* from the red parent getting into the same individual.

Red parent *eeKk* × Black parent *EEkk*
↓
1 *EeKk* kitey
1 *Eekk* self black

The appearance of only one kitey pigeon among the progeny from such a mating is definite proof that the red bird carries the gene *K*. In case the red parent is recessive for *k* only black progeny will result:

*eekk* × *EEkk*→*Eekk* black.

In either case, of course, some self red progeny might be produced if the black bird used for testing happened to be heterozygous for *E*.

TABLE 10

*Matings to test out recessive red birds for the gene K.*

| BIRD TESTED | MATING NUMBER | KITEY | BLACK | RED |
|---|---|---|---|---|
| T86E | T100 | 1 | 1 | 2 |
| T73B | T101 | 3 | 1 | 0 |
| T73A | T102 | 2 | 3 | 0 |
| T49A | T103 | 0 | 2 | 0 |
| T83C | T104 | 0 | 3 | 0 |
| T49D | T105 | 1 | 0 | 0 |
| T89B | T106 | 0 | 3 | 1 |
| T82E | T107 | 0 | 2 | 0 |
| T49J | T108 | 0 | 0 | 1 |
| T82F | T114 | 1 | 1 | 0 |

Ten of the self red birds resulting from the matings in table 9 were mated with self black birds to test them for *K*. The results are shown in table 10.

Definite proof is given in table 10 that of the ten red birds tested five, T86E, T73A, T73B, T49D and T82F, carried the gene *K*, in four cases, at least, in the heterozygous condition. The numbers are small, but the indications are that four of the other reds tested, T49A, T83C, T89B and T82E were recessive, *kk*. From the other bird tested, T49J, only one offspring was secured and it was red, thus giving no possibility of determining phenotypically whether or not it carried *K*. The principal thing to be demonstrated was that self red pigeons can carry the gene *K*, and this was done. This substantiates the statement made above that *K* is a pattern gene and can be expressed only when black pigment is in the extended form.

## Yellow and dun

In 1925 two birds were raised which showed an intimate mixture of yellow and dun in their feathers. These were T1D and an unbanded bird which was given the number T33A. Since dun is dilute black and yellow is dilute red (COLE 1914), it seemed probable that yellow and dun is the dilute form of kitiness. The gene *I* is sex-linked. Hence, any dilute (*i*) birds appearing from the mating of two intense pigeons will be females, since the female is the heterogametic sex in pigeons. Both T1D and T33A were females.

Matings were designed to test out the theory that yellow and dun is a dilute form of kitiness. Unfortunately, T1D died before any progeny were secured from her. But proof was obtained from T33A. In 1927 she was mated with a blue-barred male, T36A, in mating No. T75. Blue is a color of the intense series. From this mating there appeared among others a squab which was silver and a male. Silver is a dilute blue. From the known facts concerning the inheritance of the *I* gene (COLE 1912) it is evident that the blue male parent of this silver male was heterozygous for intensity, *Ii*, and that the yellow and dun female parent was a dilute. No dilute male would be produced if the female parent carried the dominant *I*, because each of her male offspring would receive this *I*. Any of her offspring not receiving it would be female.

The following year, 1928, T33A was mated with T3A, a self black, in mating No. T110. The object was to get the gene which was producing the mixture of yellow and dun into combination with *I* and *E*. If it were the gene *K* that was responsible for this yellow and dun condition, the combination of *K*, *I* and *E* should produce an intimate mixture of red and black.

Four offspring were obtained from mating No. T110, of which three were black and one was an intimate mixture of red and black. The appearance of this one red and black offspring from this mating is proof that T33A does carry the gene *K*.

Definite evidence was, therefore, obtained that T33A which shows an intimate mixture of yellow and dun is a dilute (*i*) and that she carries the gene *K*. The intimate mixture of yellow and dun type of coloration is the dilute form of kitiness.

### Red and blue

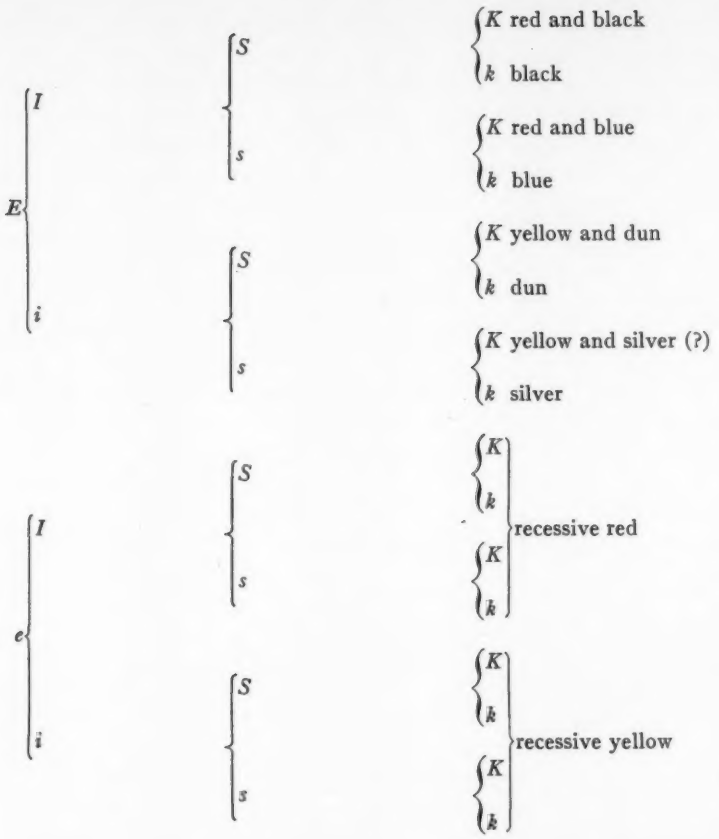
Blue is the color which results in pigeons when black pigment is clumped (LLOYD—JONES 1915). The clumping gene (*s*) has no effect on red pigment. A blue pigeon is *EIss*. The gene *K* acting on an otherwise blue pigeon should produce a blue with kitiness. 2091A appeared to be such a bird, although rather dark. Mated with blue barred mates, which are definitely clumped (*s*), she produced the following progeny: 2 black check, 1 blue black barred, 1 blue with red wing bars, and 2 showing red and blue like 2091A. One of the red and blue sons, T6B, mated with a blue barred produced 1 black check, 3 blue barred, and 3 red and blue like T6B and 2091A.

If all the pigeons used in these matings were clumped (*s*), no black check progeny would be produced since black check is epistatic to blue (JONES 1922). This pattern must have come from the red and blue parent in each case. Microscopical examination of feathers from 2091A and T6B reveals the fact that some of the black pigment is clumped and some spread throughout the greater part of the cell. There is a great deal more pigment in these feathers than there is in a blue feather from a blue black barred pigeon. Hence, both 2091A and T6B belong higher in the epistatic series than blue and are probably the result of the action of gene *K* on black check.

The exact effect of *K* on a pigeon in which the pigment is completely clumped is not known at present. The one mentioned above, which is blue with red wing bars, may be the result of this combination. A definite statement on this must await future analysis.

It seems probable, from the evidence given above, that *K* is a pattern gene which, in the dominant form, acts on black pigment whether this be spread or clumped, intense or dilute.

The following chart shows the results of the genes *E*, *I*, *S* and *K* in various combinations:




No combination *EisK* has been obtained. Hence, it is not known definitely that yellow and silver would result from this combination. But that would probably be the phenotype in case this combination of genes were obtained.

In the classifications used earlier in this paper where the relationship between black and kitiness was being studied, red and black, red and blue, and yellow and dun pigeons were all classified as kitey, since it is now evident that each of these types carries the dominant gene *K*. Likewise, black, blue, and dun pigeons were all classified as black, since each of these is recessive, *kk*.

### Effect of *A* and *K* in combination

Another pattern gene, *A*, in pigeons has also been shown (COLE and KELLEY 1919) to produce its effect only when in a bird which has the extension of black pigment, that is, an *E* bird. Thus, *A* and *K* require identical situations as working bases. The question arises, what type of color pattern will be exhibited by a pigeon which carries *E*, *A* and *K* all in the dominant condition? In mating No. T76 a dominant red (*A*) male, T25A, was mated with a kitey female, 2091A. Two progeny resulted, one of which, T76A, was black with kitiness, the other recessive red. The recessive red bird tells nothing concerning the point in question because it is a non-extended (*e*) bird. A previous mating had demonstrated that T25A was heterozygous (*Aa*). It was, therefore, necessary to apply the breeding test to the kitey progeny, T76A, to determine whether or not she carried *A* as well as *K*. Mated with a blue male she has produced two offspring each of which was black with kitiness. While this does not constitute definite proof that T76A did not carry gene *A*, it does furnish some evidence that she did not.

In mating No. T77 a kitey male, 2108A, was mated with a dominant yellow check (*A*) female, T28A. Two offspring, T77A and T77C were produced, which were dominant red males. They showed definitely the pattern produced by *A* and gave no indication of the pattern produced by *K*. The kitey parent, 2108A, was known from the results of a previous mating to be heterozygous (*Kk*). Hence, it was necessary to apply the breeding test to T77A and T77C to determine whether or not either of them carried *K*. T77A mated with a black female produced two offspring showing the pattern due to *A* and four showing kitiness, the pattern due to *K*, demonstrating that T77A carried both *A* and *K*. T77C has produced no kitey offspring.

The breeding facts given above, particularly those obtained from T77A. furnish good evidence that *A* is epistatic to *K* in pigeons.

### Variability of the pattern

The pattern produced by *K* is very variable. The proportionate amounts of the red and black pigments vary so that in some birds the feathers are almost completely black with only a sprinkling of red; whereas, in others the color is largely red. There are all degrees of the mixture between these two extremes.

Furthermore, the pattern varies with regard to the portion of the pigeon on which it occurs. The following types have been observed:

1. Those which have kitiness in all their feathers.

2. Those which have kitiness in all their feathers excepting their tail feathers.
3. Those which have kitiness in all their feathers excepting those on the head and in the tail.
4. Those which have kitiness in the wing and back feathers only.
5. Those which have kitiness in the feathers on the wings and head only.
6. Those which have kitiness in the wing and tail feathers only.
7. Those which have kitiness in the wing feathers only.
8. Those which have kitiness in the primaries only.

These regions of red and black pattern production agree in location with the pterylae, or feather tracts, on the pigeon. The pterylosis of the pigeon is shown in figure 4.

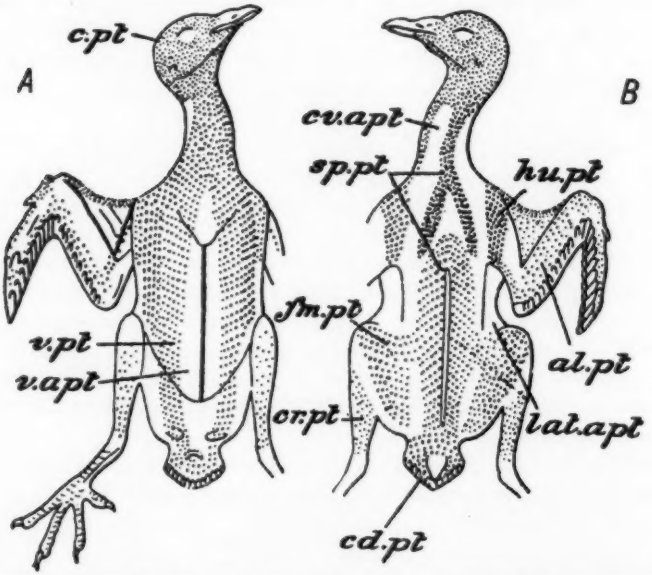



FIGURE 4.—Pterylosis of *Columba livia*. *A*, ventral; *B*, dorsal. *al. pt.* alar pteryla or wing-tract; *c. pt.* cephalic pteryla or headtract; *cd. pt.* caudal pteryla or tail-tract; *cr. pt* crural pteryla; *cv. apt.* cervical apterium or neck-space; *fm. pt.* femoral pteryla; *hu. pt.* humeral pteryla; *lat. apt.* lateral apterium; *sp. pt.* spinal pteryla; *v. apt.* ventral apterium; *v. pt.* ventral pteryla. (After NITZSCH.)

The production of the different types outlined above can be explained on the basis of the development of kitiness in certain pterylae and its failure to develop in others. The different types develop as follows:

1. The pattern is developed in all the pterylae, thus appearing in all the feathers on the pigeon.

2. The pattern is developed in all the pterylae except the caudal pteryla, thus appearing in all the feathers on the pigeon excepting the tail feathers.

3. The pattern is developed in all the pterylae except the cephalic pteryla and the caudal pteryla, thus appearing in all the feathers on the pigeon except those on the head and in the tail.

4. The pattern is developed in the alar, humeral and spinal pterylae, thus appearing in the feathers on the wings and back of the pigeon.

5. The pattern is developed in the alar, humeral and cephalic pterylae, thus appearing in the feathers on the wings and on the head.

6. The pattern is developed in the alar, humeral and caudal pterylae, thus appearing in the wing and tail feathers only.

7. The pattern is developed in the alar and humeral pterylae, thus appearing in the feathers on the wings only.

8. The pattern is developed in that portion of the alar pteryla which produces the primaries, thus appearing in the primaries only.

Some of the matings of kitey with kitey, and of kitey with non-kitey throw some light on the hereditary relationships of these different types of kitiness. Since the types of the parents are different in each mating, the matings are presented separately rather than in tabular form.

Mating No. T46 was between T3B, kitey throughout except the tail, and T3E, kitey throughout except the rump and tail. Fourteen progeny resulted from this mating, of which 6 were kitey throughout except the tail, 2 were kitey in the wings only, 3 were black and 3 were recessive red.

Mating No. T50 was between T2B and T2C, both of which were kitey throughout except the tail. This mating produced 4 offspring, which were kitey throughout except the tail, 2 which were kitey throughout except the tail and head, and 1 which was kitey in the wings only.

Mating No. T58 was between 2098A, kitey in the wings, remainder of feathers black, and 2110A, kitey throughout. The progeny were 3 kitey throughout, 2 kitey throughout except the tail, and 1 recessive red.

Mating No. T64 was between 2090A, kitey in the wings, remainder of feathers black, and 2112A, kitey throughout. This mating produced 1 offspring which was kitey throughout and 2 which were kitey throughout except the rump and tail.

In no case in any of the four matings given above were any progeny produced which had kitiness in the feathers in a pteryla in which the feathers were not kitey in one or both parents. In each mating, however, progeny were produced which had kitiness in the feathers from fewer

pterylae than did one or both parents. These facts indicate that the presence of a pattern in a certain pteryla is dominant to its absence from this same pteryla.

In mating T60, a black pigeon T2F was mated with T2A, kitey throughout. The progeny were 1 kitey throughout except the tail, 1 kitey in the wings and tail, black elsewhere, and 2 black.

T2F, black, was mated with T2B, kitey throughout except the tail, in mating T90. This mating produced 1 kitey on wings, back and breast, 1 kitey on wings only, and 6 sooty, black, and dun—not showing the pattern.

From each of these matings progeny were produced which had kitiness in the feathers from fewer pterylae than did the kitey parent. These results are in agreement with those noted above from matings of two pigeons, each of which was kitey.

There are three possible explanations of the genetic relationships of the different types of kitiness. (1) That there is a series of multiple allelomorphs determining the particular type of the pattern. (2) That there is one gene, *K*, which determines whether or not the pattern is produced and that the distribution of the pattern among the different pterylae is due to modifying genes. (3) That *K* is a variable gene.

In case a series of multiple allelomorphs is concerned, it would be expected, on the basis of the breeding facts presented above, that the members of the series for greater extent of the pattern would be dominant to those for lesser extent of the pattern. Then the mating of a pigeon with the pattern in the feathers from certain pterylae with a pigeon without the pattern would produce no offspring with the pattern in the feathers from any other pteryla. Disproof of this theory is given by mating T117, in which 2098A, kitey in the wings only, was mated with T87D, dun. One of the progeny, T117C, was kitey throughout except the tail. T117C thus had kitiness in the feathers from several more pterylae than did its kitey parent, 2098A.

The greater extension of the pattern in T117C than in 2098A can be explained by assuming that the extent of the pattern is determined by modifying genes and that the dun parent, T87D, transmitted to T117C modifying genes for greater extent of the pattern. None of the observed breeding facts are contrary to the hypothesis of modifying genes, It probably offers the correct explanation of the facts. There is a marked similarity in the breeding behavior of this pattern and of the hooded pattern in rats which was studied by CASTLE (1924).

However, the breeding facts are not of such a nature as to permit posi-

tive denial of the possibility that *K* is a variable gene, of the nature suggested by DEMEREC (1928), ANDERSON and EYSTER (1928), EMERSON (1917) and others. To decide whether the variability is due to modifying genes or to a variable gene it would be necessary to attempt to produce strains of pigeons which would breed true for different variations of kitiness. The best way to do this would be to inbreed (EAST and JONES 1919). If such true breeding strains could be established, it would be demonstrated that the variations in kitiness were caused by modifying genes, which had been gotten into a homozygous condition in each of the true breeding strains. If such true-breeding strains could not be established, it would then be time to look into the question of variable genes.

### *Studies concerning the Archangel*

#### Relationship of the pattern to black

Archangels were mated with self blacks in matings Nos. T4 and T43. The results are presented in table 11.

TABLE 11

*Archangel×Black.*

| MATING NUMBER | BLACK | BRONZE AREA | ARCHANGEL | NO CREST | CREST |
|---|---|---|---|---|---|
| T4 | 3 | 2 | 0 | 5 | 0 |
| T43 | 2 | 2 | 0 | 2 | 0 |
| Total | 5 | 4 | 0 | 7 | 0 |

No progeny showing the Archangel pattern were produced, indicating that this pattern is recessive to self black. However, four of the nine offspring did show a bronze area in the breast. This is very small in the T4 birds and rather extensive in the T43 birds. This indicates that black is incompletely dominant to the Archangel pattern. None of the $F_1$'s showed the Archangel crest on the head. It is not always noted on the description sheet whether or not the bird in question has a crest. For that reason the total number of birds used in studying the crest does not always agree with the total number used in studying the Archangel pattern.

Wisconsin mating No. 2094 was between 2014C, a black male, and 2086A an Archangel female. Thirteen offspring were produced from this mating, of which six were self black and seven black with a small bronze area. Thus the results were similar to those secured from the crosses made in Texas. With regard to the crest, however, the situation is different. Of the 2094 birds, five are described as having the crest, three as not having it, and no mention is made of it in describing the other five. No definite explanation

can be given as to why some of the 2094 birds have the crest. But in view of results which will be presented below, 2014C must have been heterozygous for the gene *C* which, in the recessive condition (*cc*), is shown to produce the crest. The pedigree of 2014C was examined as completely as possible in the record books of the Department of Genetics of the UNIVERSITY of WISCONSIN, but no ancestors were found in his pedigree in the description of which any mention was made of a condition of the head feathers which might be taken as even a resemblance to the Archangel crest. The only suggestion was found in the description of 45A, a brother to 45B which appears in the fifth generation on the sire's side of the pedigree of 2014C and in the seventh generation on the dam's side of his pedigree. 45A is described as black with a decidedly reddish cast to the breast. This condition may be the same as the bronze area noticed in the breasts of several of the hybrids between blacks and Archangels which are reported on in this paper. It might, therefore, be taken as an indication that there is some Archangel ancestry in the pedigree of 2014C. In case that is true, the gene for the crest could have been carried along in the recessive condition, masked by its dominant allelomorph, and thus reached 2014C. Then 2014C, when mated with a recessive, was able to produce progeny showing the recessive condition—the crest. Furthermore, as pointed out in section B below, crests of feathers occur on the heads of pigeons of other breeds than the Archangel. It is possible, then, that 2014C had inherited the gene for the crest from an ancestor that was not Archangel.

Black non-crested $F_1$'s from the cross of black on Archangel were mated to produce $F_2$'s. The results obtained are given in table 12.

TABLE 12

*$P_1$ Black×Archangel. $F_1$ Black×$F_1$ Black.*

| MATING NUMBER | BLACK | BRONZE AREA | ARCHANGEL | NO CREST | CREST |
|---|---|---|---|---|---|
| T38 | 8 | 1 | 0 | 5 | 3 |
| T39 | 9 | 8 | 1 | 16 | 1 |
| T67 | 0 | 1 | 1 | 0 | 2* |
| | 17 | 10 | | | |
| Observed | 27 | | 2 | 21 | 4 |
| *Calculated 15:1* | *27.1875* | | *1.8125* | | |
| *Calculated 3:1* | | | | *18.75* | *6.25* |

* Not counted in the totals because both parents had the crest.

Deviation, Archangel pattern 0.1875 ± .8793

Deviation, Crest 2.25 ±1.4604

Before discussing these results it will be well to consider also the backcross data. $F_1$'s from the cross of black × Archangel were mated with pure Archangels. The results are presented in table 13.

TABLE 13

*$P_1$ Black×Archangel. $F_1$ Black backcrossed to Archangel.*

| MATING NUMBER | BLACK | BRONZE AREA | ARCHANGEL | NO CREST | CREST |
|---|---|---|---|---|---|
| T45 | 3 | 0 | 1 | 2 | 2 |
| T72 | 2 | 0 | 0 | 1 | 1 |
| T94 | 1 | 1 | 2 | 3 | 1 |
| T95 | 2 | 1 | 1 | 4 | 0 |
| T96 | 2 | 1 | 0 | 1 | 2 |
| T97 | 0 | 3 | 1 | 1 | 3 |
| T98 | 0 | 4 | 0 | 0 | 4 |
| T118 | 0 | 2 | 0 | 0 | 2 |
| | 10 | 12 | | | |
| Observed | 22 | | 5 | 12 | 15 |
| *Calculated 3:1* | *20.25* | | *6.25* | | |
| *Calculated 1:1* | | | | *13.5* | *13.5* |

Deviation, Archangel pattern $1.75 \pm 1.5176$
Deviation, Crest $1.5 \pm 1.7537$

In both tables, 12 and 13, several pigeons are listed as being black with an area of bronze feathers on the breast. These descriptions are based on the juvenile plumage. The bronze area which occurred on the breasts of these $F_2$ and backcross pigeons was quite variable in size. In some it was very small, being much like that on the breasts of some of the $F_1$'s. In others it was much larger, including all of the parts which are bronze on an Archangel excepting the head and upper part of the neck, the region which is feathered from the cephalic pteryla. No attempt was made to divide the birds with the bronze areas on their breasts into different classes. In tables 12 and 13 they were all classed with the non-Archangel group.

According to both the $F_2$ data and the backcross data, the difference, in the juvenile plumage, between the Archangel pattern and the lack of complete development of this pattern (including both black and bronze area birds) is due to two genes, both of which must be homozygous recessive in order that the Archangel pattern may be produced. These genes have been given the symbols *P* and *Q*. Since there is 1 double recessive individual out of 16 in the $F_2$ of a dihybrid, the expected $F_2$ ratio is 15 not Archangel: 1 Archangel. On the basis of this same explanation a ratio of 3 not Archangel:1 Archangel is expected from the backcross of $F_1$ on Archangel.

The results obtained in both the $F_2$ and the backcross are very closely approximated by the results calculated on the basis of the explanation given above, as shown in tables 12 and 13. In the case of the $F_2$ ratio there is a slight excess of the Archangel pattern, but the deviation is much smaller than its probable error. In the backcross ratio there is an excess of blacks, but the deviation from the expected ratio is not significant, being only slightly larger than its probable error.

Very few of the $F_2$ and backcross birds with the bronze areas on their breasts were saved until they had passed through molt. This is an unfortunate circumstance because some of those which were saved had very good Archangel patterns in the adult plumage. It would be instructive to know what would have happened in the case of each of these birds. In the case of the backcross birds, T98E and T98D, each had a good Archangel pattern following molt. T98C, however, retained the black on the head and neck. Likewise, only three $F_2$ bronze area birds lived to pass through molt. These were $T38A_2$, $T39A_2$ and $T39F_2$. Each of these birds was described as having a small bronze area in the breast in the juvenile plumage. In the adult plumage $T38A_2$ has no bronze in the breast and the other two birds have still a very small amount of bronze in this region. Thus, some of the bronze area birds changed to the Archangel pattern following molt, whereas others did not.

The question arises, to what agency can this difference between juvenile and adult plumage be attributed? Two possibilities suggest themselves. The first is that of the delayed action of the gene. It is a well-known fact that horses which will be gray at maturity are black when foaled and become gray only after shedding the first coat of hair. Likewise, it is known that many tow-headed children become darker headed as they grow older.

PUNNETT (1923) shows that as regards henny-feathering in male chickens, the juvenile plumage may show an intermediate condition, whereas, after molting complete henny feathering may be shown. These intermediates, when bred from, behave as hennies and may produce sons which are almost completely henny. He says, "The facts seem to suggest that in some cases where the cock is heterozygous the inhibiting substance is not produced in sufficient bulk at a stage sufficiently early to suppress the developing cock plumage completely. Later on, as the testes increase in size, the substance is produced more freely, and almost, or quite complete hen-feathering results."

MORGAN, BRIDGES and STURTEVANT (1925) writing on the action of genes say, "Whether the genes are active all the time, or whether their

activity is correlated with certain phases of development, we do not know at present and can scarcely even surmise."

The explanation of the change in pattern on this basis is that there is one gene which accounts for the difference between Archangel pattern and not Archangel pattern in pigeons. This gene must be double recessive in order that the Archangel pattern may be produced, that is, that the feathers on the bronze parts of the Archangel may be prevented from producing black pigment. The action of these genes is delayed in certain regions, particularly in the cephalic pteryla, in some of the recessive birds, permitting the feathers in the juvenile plumage in these regions to become black. Throughout later life, however, these genes are active and the bird carrying them exhibits the Archangel pattern following molt. In order to account for the production of some birds with the Archangel pattern in their juvenile plumage in the $F_2$ and backcross generations, it is necessary to assume that in some cases these genes were active sufficiently early in development to produce this pattern in the juvenile plumage.

The other explanation is that there is one gene, *P*, which accounts for the difference between Archangel and not Archangel, and that the double recessive, *pp*, is modified in its action in the juvenile plumage by another gene, *Q*, which prevents the action of *pp* in the feathers from the head pteryla. But *Q* acts only in the juvenile plumage. It is inactive in the older pigeon. Hence, *ppQQ* and *ppQq* pigeons are black with a bronze area on the breast in their juvenile plumage, whereas in their adult plumage they exhibit the Archangel pattern. The expectation on this basis is that the $F_2$ generation would show a ratio of 15:1 in the juvenile plumage and of 3:1 in the adult plumage. The backcross ratio would be 3:1 in the juvenile plumage and 1:1 in the adult plumage.

There are no breeding facts available to aid in deciding which possibility is correct. The writer is inclined, however, to accept the latter. It seems probable that if the gene were delayed in its action it would be delayed in all cases so that on that basis no pigeons with the Archangel pattern in the juvenile plumage would be produced in the $F_2$ or backcross generation. But some were produced. To account for them it would be necessary to assume that the same gene is active at different stages of development in different individuals, which may be possible but is not probable. The second hypothesis, on the other hand, accounts for the production of both types in the $F_2$ and backcross generations and in the ratios observed.

Wisconsin mating No. 2216 was between 2087A, an Archangel male and 2094E, a dun checker female, which was an $F_1$ from a black × Archangel cross. It was, therefore, a backcross similar to those given in table 13.

Two progeny resulted from this mating, both of which were black. This is not out of line with the expected ratio of 3 not Archangel:1 Archangel.

Mating No. T54 was between 2094C, an $F_1$ from a black×Archangel cross, and 2014C, a self black. This mating was a backcross of the $F_1$ on black. Seven offspring resulted from this mating, all of which were self black as was to be expected on the basis of the explanation offered above.

The most probable explanation of the occurrence of a black with a small area of bronze on the breast is that *P* is incompletely dominant. The other suggestion was offered that there is a gene for the production of the bronze area. If this were true, either one type or the other, the self black or the black with bronze area should breed true. But each type has produced the other. Mating No. T38 was between two self black $F_1$'s. This mating produced eight self black and one black with bronze area. Mating No. T39 was between two $F_1$'s, each of which was black with a bronze area. The progeny were nine self black and eight black with bronze area.

BESSMERTNAYA (1928) has reported a dominant gene for the Archangel pattern. METZELAAR (1926) also states that the Archangel pattern is of a dominant type. But, as noted in this paper, the results obtained both in Wisconsin and in Texas are not in accord with this theory. The data available demonstrate, without exception, that the Archangel pattern is recessive.

No attempt has been made thus far to combine the Archangel pattern with clumped black pigment or with dilute black pigment. LAVALLE and LIETZE (1905) and METZELAAR (1926) describe Archangels that are blue in the regions which are usually black. It would seem that these are Archangels with clumped pigment, *ss*. No descriptions have been seen of Archangels with dun or silver pigment, but it seems probable that such types could be produced by the proper recombinations of the genes. LAVALLE and LIETZE also describe Archangels which are white in the regions which are usually black.

## Inheritance of the crest

The data presented in tables 11, 12 and 13 show that the Archangel crest is inherited as a simple Mendelian recessive to the usual smooth feathered condition on the head. The gene for this difference is given the symbol *C*. A pigeon without the crest is either *CC* or *Cc*. One which has the crest is *cc*. The agreement of the observed and calculated ratios in both the $F_2$ and the backcross generations is good. In the $F_2$ there is an excess of birds without the crest, but in the backcross the excess over the calculated number is among the birds which have the crest. The deviations, small though they are, thus tend to offset each other.

These results are comparable with those of CHRISTIE and WRIEDT (1923) who studied the inheritance of the Rundkappe (round cap) in the Norwegian Petent pigeon. In the Rundkappe of this breed the feathers on the back of the head from ear to ear are reversed and form a sort of cap or hood. The Rundkappe was found to be due to one recessive gene which they call $r_u$. Since the behavior of $r_u$ is the same as that of $c$ it is probable that the two symbols represent the same gene. STAPLES-BROWNE (1906) reported that the shell of the Nun pigeon is inherited as a simple Mendelian recessive to the smooth headed condition. This shell is similar to the Rundkappe, the feathers from ear to ear being reversed, forming a cap or hood. The crests on the heads of some of the segregates from Archangel crosses are wider in extent than those on typical Archangels. These crests in some cases reach from ear to ear. A crest of feathers on the head is typical for a Red Crested Helmet pigeon. A pigeon reputed to be a Red Carneau, shown in the pigeon show at Brenham, Texas, in February, 1929, which was judged by the writer, had a crest of feathers on its head which resembled very closely the crest on the head of an Archangel. It may be that all head caps or crests on pigeons are due to the same gene, and that the particular shape which the crest assumes is determined by modifying genes.

### Relationship of the pattern to recessive red

The relation of the Archangel pattern to recessive red has been studied, but very unsatisfactorily. For some reason or other, matings made between these two types have not done well and very few offspring have been produced. Only two matings have produced any at all. The results of these are given in table 14.

TABLE 14

*Archangel×Recessive Red.*

| MATING NO. | BLACK | ARCHANGEL |
|---|---|---|
| T 88 | 3 | 1 |
| T111 | 2 | 0 |

Theoretically all of the progeny from these matings should be black if the parents were homozygous. The Archangel parent furnishes the gene $E$ for the extension of black pigment and the red parent furnishes the genes $P$ and $Q$ which make self color dominant over the Archangel pattern. The Archangel produced in T88 can be accounted for by assuming that the red parent, 1875C, was heterozygous for $P$ and $Q$.

All of the birds produced from these two matings with the possible

exception of T111G were kitey. This kitiness could have come from the red parent. It is known from the results of another mating that 1875C, the red used in T88, was heterozygous for *K*, the gene which produces kitiness. As stated above in the description of the Archangel, a great many pigeons of this breed have red flecking in their wings. It has been determined that this red flecking is dominant to self black. It is inherited in the same manner as the kitiness described earlier in this paper, and undoubtedly is identical with it. The Archangel used in each of the matings under consideration had kitiness in the wings. Hence, the kitiness in the progeny was inherited from the Archangel parent.

The Archangel Fancy reports the occasional production of an "all bronze" Archangel. This is undoubtedly due to the segregation of a recessive. Whether or not this "all bronze" Archangel is a recessive red cannot be stated because none of the "all bronze" Archangels have been bred, or, even observed, by the writer of this paper. It would seem, however, to be a good suggestion for a working hypothesis that the production of these "all bronze" Archangels is due to the segregation of *ee* birds.

### Effect of *A* in combination with the Archangel pattern

Two matings were made between Archangels and pigeons carrying the gene *A*. In one of these, T44, a yellow bar (*A*) male, T29A, was mated with an Archangel female, T16A. Five progeny were produced, two dominant reds and three dominant yellows. These all show very definitely the pattern due to *A* and no sign of the Archangel pattern. This is as was to be expected because *A* is dominant to self black, whereas the Archangel pattern is recessive to self black.

Mating No. T79 was between an Archangel male, T21A, and a dominant red female, T31A. Two offspring were produced. T79B, a male, was dominant red. T79A, a female, showed a fairly good Archangel pattern. Only the male offspring from this mating would receive the gene *A* because it is sex-linked (COLE and KELLEY 1919). These two matings demonstrate that whenever gene *A* is in combination with the Archangel pattern, the pattern due to the action of *A* is produced.

### Dark bronze and light bronze

As pointed out in the description of the Archangel, there are two distinct types known as the dark bronze and the light bronze. Matings were made between birds of the two types with a view of studying the genetic relationship between them.

Mating No. T41 was between T14A, a dark bronze Archangel male, and T15A, a light bronze Archangel female. This mating produced one typical dark bronze Archangel and four birds which showed the Archangel pattern with a shade of bronze intermediate between dark bronze and light bronze, but approaching rather closely to the dark bronze.

Mating No. T80, which was between T18A, a light bronze Archangel male, and 2084A, a dark bronze Archangel female, produced one light bronze Archangel offspring. The bronze on this pigeon, T80A, is, however, darker than the shade desired in light bronze Archangels. T80A was mated in T94 with a black, $2094C_2$. A two generation pedigree of the T94 birds is as follows:

T94
- $2094C_2$ black
  - 2014C black
  - 2086A dark bronze Archangel
- T80A light bronze Archangel
  - T18A light bronze Archangel
  - 2084A dark bronze Archangel

Two of the progeny, T94A and T94C, were light bronze Archangels. Two others, T94G and T94H, were black with large light bronze areas on the breasts. T94B was black check with a small bronze area, the shade of which was not noted. T94D was black.

Mating No. T42 was between two dark bronze Archangels, T21A and T22A. This mating produced three dark bronze Archangels, one light bronze Archangel, and two Archangels with an intermediate shade of bronze.

These matings do not solve the problem of the relationship between the two types of bronze. No further analysis has, however, been made. The shade of bronze produced was found to be very variable. It was impossible to describe the shade of bronze definitely without a set of color standards, which were not available. Grayish areas appeared in the throats of some of the birds.

An attempt was made to classify the progeny from the above mentioned matings on the basis of their juvenile plumage. Not many of them were carried over to their second year. It has now been observed that the adult plumage assumed after molt is much more definitely either dark or light bronze than is the juvenile plumage. Further study of this problem will have to be made on the basis of adult plumage rather than of juvenile plumage.

METZELAAR (1928) has suggested that the difference between the two types of Archangels is due to a dominant dilution gene which in the homozygous dominant or heterozygous condition produces the light bronze and in the homozygous recessive condition produces the dark bronze. If this were true, crosses between the two types should produce only light bronze progeny or equal numbers of both types, depending on whether the light bronze parent were homozygous or heterozygous for the gene responsible for dilution. As noted above, mating T80 produced one light bronze offspring, T80A, which in turn produced light bronze offspring but no dark bronze in T94. But mating T41 produced five offspring, not one of which was light bronze. Granting that the light bronze parent used in T41 was heterozygous, the production of five recessives (dark bronze) and no dominants (light bronze) from such a mating would happen due to chance alone only once out of thirty-two times. Thus, the odds are seen to be against the suggestion of the dominant diluter.

If dark bronze were the recessive condition, matings between dark bronze birds should produce no light bronze offspring. However, as indicated above, such a mating, T42, did produce a light bronze offspring, a fact which is not at all in accord with the theory of a dominant dilution gene. METZELAAR had suggested previously (1926) that the dark bronze was an intense (*I*) condition, whereas the light bronze was dilute (*i*). This was easily checked up on because the relationship of intense and dilute pigeons is known definitely (COLE 1912). Intense is dominant to dilute and the gene *I* is sex-linked.

In mating No. T43 a light bronze Archangel male, T18A, was mated with a dun female, T27A. Dun is a dilute color. The result was four black offspring, two males, one female and the sex of the other is not known. Black is an intense color. These T43 birds must necessarily have received the *I* from their light bronze Archangel parent, T18A. In case T18A is homozygous for *I* nothing but intense progeny would be expected from this mating.

Mating No. T44 was the reciprocal of T43. A yellow bar (dilute) male, T29A, was mated with a light bronze Archangel female, T16A. From this mating there were secured four progeny, of which the sex has been definitely determined. These consisted of two dominant red (intense) males, and two dominant yellow (dilute) females. Here again the gene *I* must necessarily have been received from the light bronze Archangel parent. These results agree with the expectation that when a dilute male is mated with an intense female the male offspring will be intense and the female offspring dilute. Adequate proof is furnished from these two matings, T43

and T44, that the light bronze condition is not due to the sex-linked dilution gene *i*.

*Black feathers in recessive reds*

Very occasionally a recessive red pigeon has one or a very few black feathers. This condition has been observed in 1057A, 1875C, T49D and T55C. Since red is recessive to black this is an unusual condition. A recessive red pigeon is *ee*. A black is either *EE* or *Ee*.

Only one black feather appeared in 1875C. This was found among the coverts of the left wing, which were red. This feather had a sprinkling of red throughout, which was undoubtedly due to the gene *K*, since it has been proved, as outlined earlier in this paper that 1875C carried *K*.

Also, only one black feather appeared in T49D. This bird was recessive red but had a great deal of white on it. The one black feather appeared on the rump in the midst of an area of white feathers.

In T55C an area of fourteen black feathers occured just under the right shoulder joint, and another area of three black feathers occurred on the front part of the humeral portion of the right wing.

An area of several, probably six or eight, black feathers, occurred on the back of 1057A. This group of feathers was completely surrounded by red feathers.

T49D and 1875C have been used in matings, but only with black mates. They have produced no recessive red progeny. Hence, no data are available to indicate whether or not the appearance of the black feathers is hereditary. This condition cannot be considered as a secondary sexual character because 1057A was a female, whereas the others were males.

A number of analogous situations have been reported in several different animals. In some cases the aberrant color appearing has been of a type that is normally dominant to the color exhibited by the remainder of the animal or organ. In other cases the aberrant color has been of a recessive type.

PINCUS (1929) reports three mice which were heterozygous for black and chocolate (*Bb*) each of which was black with a small area of chocolate in its pelage. He believes that these chocolate areas may be accounted for either by non-disjunction resulting in cells from which the chromosome carrying *B* is missing, or by a temporary reversal of dominance of such a nature that pigment deposition in these areas was governed by the *b* (chocolate) gene.

A blue area on a black rabbit was reported by CASTLE (1929). This condition was transmitted to the offspring in certain instances. The rabbits in question were heterozygous for intense and dilute, *Dd*. CASTLE

says there are three possible explanations of this blue patch: 1. A somatic mutation has occurred resulting in loss of the *D* chromosome from an area of the skin, in which *d* is left free to express itself. 2. Reversal of dominance of *D* over *d* in this particular area. These two possibilities are rejected by CASTLE. 3. The explanation to which CASTLE is inclined is that the supposed gene *D* in this case is really a mosaic and that some of the genomeres of this gene are *d*. As a result of continued somatic cell divisions, some of the daughter cells come to be *d* in all or nearly all of the genomeres of this mosaic gene and produce the dilute color, in this case blue.

PATTERSON (1928) has produced a large number of Drosophila after treatment with X-rays in which the eyes which would normally be red have some white ommatidia. He has explained the production of these white ommatidial areas in two ways. In some cases there has been a gene mutation from *W* (red) to *w* (white) in the somatic cells which were ancestral to these particular ommatidia. In other cases the X-ray has produced chromosome abnormalities, involving the loss of the X-chromosome, or at least that part of it which carries the dominant gene *W*.

The foregoing cases, each of which involves the production of a recessive area, are open to several explanations. Not all of the explanations will apply, however, in case the aberrant area is of a dominant type. The loss of a chromosome or a part of a chromosome would not change the type because the homologous chromosome carries the same gene as does the one that was lost. Reversal of dominance does not apply because the gene for the aberrant dominant color is not in the normal cell, hence cannot be reversed with. The genomere theory might account for the facts in case a preponderance of recessive genomeres dominated a few dominant genomeres. During somatic cell divisions the relative proportions of the dominant and recessive genomeres might become changed to such a proportion that more dominant than recessive genomeres were in the gene and this gene became dominant over its recessive allelomorph. Such a change in the gene is essentially a gene mutation. The remaining explanation is that of gene mutations, which is just as applicable to the production of dominant aberrant areas as to the production of recessive mutant areas. This explanation has been offered as the most probable one for the cases given below.

PATTERSON has produced, by means of X-ray treatment, a dominant somatic mutation in Drosophila. This is a case in which apricot eye color has mutated to near red. It is an area on one eye consisting of about twenty-five facets.

TIMOFEEFF-RESSOVSKY (1929), also using X-ray treatment, reports the production of three dominant somatic mutations in some of the facets of the eyes of Drosophila. One case was from white to red, another from eosin to red, and the third from white to a light red allelomorph.

The occurrence of a black spot on a red steer has been reported by the writer (HORLACHER 1928).

WRIGHT and EATON (1926) report the occurrence of a guinea pig which had two red agouti (intense) areas on an otherwise yellow agouti (dilute) pelage. Both of his parents were dilute. He transmitted the dominant mutant gene for intensity to about one-third of his progeny. WRIGHT and EATON offer the explanation that this guinea pig was $c^d c^d$ initially but that one $c^d$ mutated to $C$ in a cell ancestral to part of the soma and also to part of the germinal epithelium.

The appearance of black feathers in recessive red pigeons can be explained on the same basis as the appearance of dominant aberrant areas in other animals. A recessive red pigeon is *ee*. A black pigeon is either *EE* or *Ee*. The explanation is that the pigeon is recessive red, *ee*, originally. A mutation occurs from *e* to *E* in one of the somatic cells. This cell and its descendants are *Ee*. Feathers developing from cells descended from this cell will be black as a result of the action of *E*. In case only one feather is black, as in 1875C and T49D, the mutation occurred late enough in development so that only one feather papilla received the mutant gene *E*. In case several feathers are black, as in 1057A and T55C, the mutation occurred in a cell which was ancestral to several feather papillae, each of which received the mutant gene *E*.

## SUMMARY AND CONCLUSIONS

1. This paper is a study of the inheritance of two different red and black color patterns in pigeons and of their relationships to the various self colors. These patterns are:

A. Kitiness. An intimate mixture of red and black over the entire bird.

B. Archangel. The pattern found in the Archangel pigeon in which the two colors are confined to definite areas of the bird.

2. The inheritance of the crest on the Archangel was studied also.

3. Each of these patterns is produced by a pattern gene or genes which produce their effects only when they have as a basis extended black pigment (*E*).

4. Kitiness is produced by a dominant gene which has been given the symbol *K*.

5. Recessive red pigeons may carry *K*, but it is not expressed because the black pigment is not extended (*e*).

6. The effect of the dilution gene (*i*) on a pigeon which would in the intense (*I*) series show an intimate mixture of red and black is to produce an intimate mixture of yellow and dun.

7. The experimental results which are given indicate that the effect of the clumping gene (*s*) on a pigeon which would in the spread condition (*S*) show an intimate mixture of red and black is to produce an intimate mixture of red and blue.

8. Evidence is presented which indicates that the gene A is epistatic to the gene *K*.

9. The pattern produced by *K* is very variable.

10. The Archangel pattern is recessive to self black. It differs in expression, in regard to both the extent of the pattern and the shade of bronze, in the juvenile plumage and the adult plumage. There apparently are two genes concerned, which have been designated *P* and *Q*. *pp* produces the Archangel pattern, but *Q* prevents it from being produced on the head and neck in the juvenile plumage.

11. Matings between Archangels and pigeons carrying the dominant pattern gene *A* produce progeny which show the pattern due to *A*.

12. The difference between dark bronze and light bronze Archangels is not due to the intensity gene *I*.

13. The crest on the head of the Archangel is inherited as a simple Mendelian recessive. The gene for this has been designated as *C*.

14. Four examples are cited of recessive red pigeons which had one or more black feathers. This condition is explained as being due to a dominant somatic mutation.

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# TRANSLOCATIONS INVOLVING THE THIRD AND THE FOURTH CHROMOSOMES OF *DROSOPHILA MELANOGASTER**

T. DOBZHANSKY

*California Institute of Technology, Pasadena, California*

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## INTRODUCTION

The chromosomal aberrations known in Drosophila as well as in other animals and in plants may be divided in two classes. The first class comprises aberrations involving whole chromosomes or groups of whole chromosomes. Polyploidy and non-disjunction are the most frequently observed phenomena of this kind. The study of the chromosomal aberrations belonging to this class has given much information concerning the role of chromosomes in general and the role of individual chromosomes in particular in the transmission of hereditary characters. The most clear and convincing proof that the chromosomes are carriers of hereditary material is that secured by the study of non-disjunction of the first and of the fourth chromosomes of Drosophila (BRIDGES 1916, 1921). The aberrations involving sections of chromosomes constitute the second class. Here belong translocation, duplication, inversion and deficiency of section of a chromosome (see BRIDGES 1923). These phenomena are comparatively little known, although they furnish favorable material for the investigation of the distribution of hereditary material within the chromosomes. Translocations seem to be especially valuable for the study

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of the problem just mentioned because they are most likely to produce differences in the genetical behavior of some characters together with visible alterations of the chromosomes.

The term "translocation" was proposed by BRIDGES (BRIDGES 1923, MORGAN, BRIDGES, STURTEVANT 1925) "for cases in which a section of chromosome is removed from its normal location but is present in an abnormal location." Theoretically, a section removed from its normal location in one chromosome may become attached either to a new locus in the same chromosome or to another chromosome. Both intrachromosomal and interchromosomal translocations are, therefore, possible. Most of the translocations described so far (BRIDGES 1923; MULLER and PAINTER 1929; PAINTER and MULLER 1929; also the translocations described in the present paper) are interchromosomal, but DUBININ (1929) recently found also an intrachromosomal translocation.

In the case of the "Pale" translocation described by BRIDGES (1919, 1923), a section of the second chromosome carrying the genes from plexus to speck was broken off and became permanently attached to the third chromosome near the gene ebony. Flies heterozygous for the translocation, that is, carrying one deficient second chromosome and one third chromosome with the section of the second attached to it, are nearly normal in appearance but give abnormal genetical results when crossed to flies free from the translocation. Four kinds of zygotes are produced in such a cross: (1) carrying both the deficient second chromosome and the third chromosome with the attached section, (2) the deficient second chromosome and the normal third, (3) a normal second and the third with the attached section, (4) normal second and third chromosomes. Zygotes 1 and 4, representing the parental combinations of the chromosomes, give flies heterozygous for the translocation and normal flies respectively. Zygotes 2 and 3 represent the recombinations of the parental chromosomes. Zygotes 2 die; zygotes 3 sometimes survive, but give rise to flies abnormal in appearance and weak in constitution because they carry a duplication for a section of the second chromosome. The elimination of zygotes 2 and the partial elimination of zygotes 3 result in an apparent linkage of the genes belonging to the second and the third linkage groups.

The apparent linkage observed in the case of the "Pale" translocation suggests a method of finding translocations in general. Each of the chromosomes of a fly may be marked by appropriate genes. If such a fly carries no translocation, all the possible recombinations of the "marking" genes must be present in its progeny because of the free recombination of the parental chromosomes in the gametogenesis. However, if a fly with

"marked" chromosomes carries a translocation involving any two of the chromosomes, a linkage of the genes localized in those chromosomes will be observed, Hence, some of the expected classes of the offspring will be absent, or will manifest somatic peculiarities due to the disturbance of the normal genic balance.

The "Pale" translocation arose spontaneously; the same is true in respect to a few other cases of translocations (see MORGAN, BRIDGES, STURTEVANT 1925, pp. 178–179). The frequency of the spontaneous origin of translocations seems to be, however, so low as to make an attempt to secure them at will impracticable. The production of translocations in comparatively large numbers became possible only when MULLER (1928a, 1928b) and WEINSTEIN (1928) discovered that the frequency of translocation is very considerably increased in the progeny of flies treated by X-rays.

Recently MULLER and PAINTER (1929) and PAINTER and MULLER (1929) have published a preliminary account of their cytological and genetical investigations on the translocations involving different chromosomes of *Drosophila melanogaster*. Most of their conclusions coincide with the conclusions drawn in the present paper on the basis of my own material.

The present work was done in 1928 and 1929 at the MARINE BIOLOGICAL LABORATORY, Woods Hole, and at the CALIFORNIA INSTITUTE OF TECHNOLOGY, Pasadena. Preliminary accounts of the results have already been published (DOBZHANSKY 1929a and 1929b).

The author wishes to express his gratitude to Doctor T. H. MORGAN, Doctor A. H. STURTEVANT and Doctor C. B. BRIDGES for their invaluable advice, suggestions and criticism without which the present work could hardly have been done. The author is obliged to Miss E. M. WALLACE for the finishing of the drawings accompanying this paper.

## ORIGIN OF THE TRANSLOCATIONS

Males heterozygous for the second-chromosome dominant gene Bristle ($B_1$ - short bristles), and the third-chromosome dominant gene Dichaete ($D$-wings spread, anterior dorsocentral bristles missing) were treated by X-rays. The age of the flies at the moment of the treatment was not more than 14 hours from the emergence from the puparia. A glass vial was filled with cotton to within about 1½ cm from the opening; flies were placed on this cotton, and the opening of the vial closed by a cotton stopper. The vial was placed vertically, the opening up, under the target of a broad-focus Coolidge tube, so that the flies were at 16 cm from the target. Between the vial and the tube an aluminum plate of about 1 mm thickness

was inserted. The tube was operated by a current of 50 peak kilovolts and 5 milliamperes during 48 minutes. As seen from these data, the dosage of X-rays used is approximately equal to that applied by MULLER (1928) in his experiments and called "t-4"

The treated males were crossed in individual cultures to untreated females having attached X-chromosomes homozygous for the sexlinked gene yellow (*y*-yellow body color) and homozygous for the fourth-chromosome recessive gene eyeless$^2$ ($e_y^2$-eyes small, occasionally absent). Of the 57 cultures in which the fathers were treated, 18 cultures gave no offspring. Of the 10 control cultures (in which untreated males and females of the same genetical constitution as in the treated series were bred) only one culture was sterile. The average number of offspring per culture in the treated series was markedly lower than in the control series. Evidently the dosage of X-rays was sufficient to produce a significant decrease of fertility in the treated males.

In the $F_1$ generation all the expected classes of offspring appeared in each of the cultures of the treated as well as of the control series. These classes are:

| | | | | |
|---|---|---|---|---|
| Females: | *y*, | *yB*$_l$ | *y*D, | *y B*$_l$ *D* |
| Males: | +, | *B*$_l$, | *D*, | B$_l$ *D*. |

Although no particular care was paid to the detection of mutations, some were found in the cultures of the treated series, but none in the control series. Two of the mutations found were extreme allelomorphs of forked, one was a dominant (lethal when homozygous) allelomorph of vestigial (phenotypically like Beaded), and one was an extreme Minute (locus not determined). Besides the mutations, there were found nine individuals which were mosaics having one-half or one-quarter of the body Haplo-IV eyeless. These mosaics were certainly due to the elimination of the treated fourth chromosome in the cleavage of the eggs from which the mosaic individuals developed. The grand total of flies examined in the treated series was 2420, and in the control series - 1067.

Let us consider the genotypical constitution of the *B*$_l$ *D* males which appeared in the cultures of the treated series. They received a treated X-chromosome, a treated second chromosome carrying *B*$_l$, a treated third chromosome carrying *D*, and a treated fourth chromosome carrying the normal allelomorph of $e_y$ from their fathers. On the other hand, they received an untreated Y-chromosome, an untreated second and third chromosome carrying normal allelomorphs of *B*$_l$ and *D* respectively, and an untreated fourth chromosome carrying $e_y$ (The allelomorph of $e_y$

used throughout the present study is $e_y^2$. However, for sake of simplicity, the symbol $e_y$ will be used from now on.) from their mothers. In other words, all the four pairs of chromosomes of these males are "marked" by different characters, namely: sex, $B_l$ $D$ and $e_y$.

From the treated series 153 $B_l$ $D$ males and from the control series 26 $B_l$ $D$ males were crossed in single-pair cultures to untreated females homozygous for $e_y$. The progeny of this cross normally consists of 16 classes of offspring representing all the recombinations of the four "marking" characters mentioned. These classes are:

Females: $+$, $B_l$, $D$, $e_y$, $B_l$ $e_y$, $D$ $e_y$, $B_l$ $D$, $B_l$ $D$ $e_y$,
Males $+$, $B_l$, $D$, $e_y$, $B_l$ $e_y$, $D$ $e_y$, $B_l$ $D$, $B_l$ $D$ $e_y$.

However, if a parental $B_l$ $D$ male carries a translocation involving any two chromosomes, the individuals of some of these 16 classes of offspring may be expected either to be missing, or to possess visible external abnormalities. For instance, if the male carries a translocation involving the treated second and third chromosomes, no $D$, $B_l$, $D$ $e_y$ and $B_l$ $e_y$ individuals of either sex are expected in his progeny. Likewise, if such a male carries a translocation involving the treated second and X-chromosomes, no $B_l$ males and no non-$B_l$ females are expected in his progeny (or, $B_l$ males and non-$B_l$ females may occur, but be abnormal in appearance).

Of the 144 cultures of the treated series, 23 cultures were sterile; of the 26 control cultures only 2 were sterile. Furthermore, 112 cultures of the treated series and 24 cultures of the control series produced normal progenies, that is, in the overhelming majority of these cultures all 16 expected classes of offspring were present in approximately equal numbers. Only two of these "normal" cultures produced too small a number of offspring, and failed, therefore, to show one of the expected classes. Table 17 presents the summary of the counts observed in the "normal" cultures of the treated series.

The results obtained in the remaining nine cultures of the treated series are presented in table 1. In five of these cultures (No. 1271, 1318, 1319, 1407,1425) eight of the expected classes, representing the recombination of $D$ and $e_y$, are missing. This fact suggests that a translocation involving the third and the fourth chromosomes is present in each of these cultures. In cultures 1223, 1239, 1289 and 1401, eight classes which represent the recombinations of $B_l$ and $D$ are missing, suggesting the presence of translocations involving the second and third chromosomes.

The results obtained in these nine cultures are, indeed, so strikingly different from the results obtained in all the "normal" cultures, that no

TABLE 1

*Cultures showing linkage of genes located in different chromosomes.*

| CULTURE NUMBER | STRAIN | TRANSLOCATION INVOLVING CHROMOSOMES | WILD TYPE ♀ | WILD TYPE ♂ | $Bi$ ♀ | $Bi$ ♂ | $D$ ♀ | $D$ ♂ | $e_v$ ♀ | $e_v$ ♂ | $Bi\,D$ ♀ | $Bi\,D$ ♂ | $D\,e_v$ ♀ | $D\,e_v$ ♂ | $Bi\,e_v$ ♀ | $Bi\,e_v$ ♂ | $Bi\,D\,e_v$ ♀ | $Bi\,D\,e_v$ ♂ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1223 | A | II–III | 28 | 28 | .. | .. | .. | .. | 24 | 26 | 22 | 24 | .. | .. | .. | .. | 15 | 21 |
| 1239 | B | II–III | 22 | 16 | .. | .. | .. | .. | 20 | 20 | 28 | 21 | .. | .. | .. | .. | 14 | 19 |
| 1289 | C | II–III | 4 | 5 | .. | .. | .. | .. | 8 | 4 | 9 | 14 | .. | .. | .. | .. | 9 | 6 |
| 1401 | D | II–III | 3 | 8 | .. | .. | .. | .. | 7 | 7 | 2 | 4 | .. | .. | .. | .. | 4 | 12 |
| 1271 | a | III–IV | .. | .. | .. | .. | 18 | 12 | 11 | 21 | 19 | 18 | .. | .. | 10 | 12 | .. | .. |
| 1318 | b | III–IV | .. | .. | .. | .. | 14 | 13 | 20 | 15 | 21 | 13 | .. | .. | 12 | 7 | .. | .. |
| 1319 | c | III–IV | .. | .. | .. | .. | 19 | 15 | 9 | 13 | 22 | 19 | .. | .. | 26 | 17 | .. | .. |
| 1407 | d | III–IV | .. | .. | .. | .. | 13 | 7 | 12 | 14 | 10 | 11 | .. | .. | 17 | 12 | .. | .. |
| 1425 | e | III–IV | .. | .. | .. | .. | 24 | 19 | 12 | 15 | 17 | 17 | .. | .. | 13 | 15 | .. | .. |

doubt is possible in classifying a given culture as belonging to the "normal" or to the "abnormal" class.

Each of the nine cultures just described served as the progenitor of a separate strain, in which the abnormal genetical situation present in these cultures has been maintained by appropriate breeding. These strains are designated by the characters of the alphabet, as shown in table 1. The present paper deals only with the results of the investigation of the translocations involving the third and the fourth chromosomes. The translocations involving the third and the second chromosomes will be described in a separate paper.

## WORKING HYPOTHESES

The genes $D$ and $e_v$ are localized in the third and in the fourth chromosomes respectively. Nevertheless, there is observed a complete linkage between $D$ and $e_v$ in five cultures shown in table 1. The first question concerning the nature of this abnormality is whether the causes which prevented the free recombination of $D$ and $e_v$ in these five cultures are hereditary.

Dichaete non-eyeless males from each of these five cultures were crossed separately to their eyeless sisters. Only $D$ and $e_v$ (no wild-type and no $De_v$) offspring were produced (table 2). In the next generation $D$

males were crossed to unrelated eyeless females; again only *D* and $e_y$ individuals were produced. The linkage of *D* and $e_y$ is, therefore, permanent, and Dichaete individuals transmit the cause producing this linkage to their Dichaete progeny. This fact gives a simple method of maintaining stocks of the translocations: *D* males from the stock cultures of the translocations are crossed in each generation to their $e_y$ sisters or to unrelated $e_y$ females.

TABLE 2

*Progeny of Dichaete-flies (carrying translocations) when crossed to eyeless.*

| TRANSLOCATION | EYELESS ♀ × DICHAETE ♂ | | | | | DICHAETE ♀ × EYELESS ♂ | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *D* | + | $De_y$ | $e_y$ | TOTAL | *D* | + | $De_y$ | $e_y$ | TOTAL |
| a | 480 | .. | .. | 424 | 904 | 1080 | 2 | 2 | 915 | 1999 |
| b | 349 | .. | .. | 324 | 673 | 818 | 1 | 2 | 689 | 1510 |
| c | 344 | .. | .. | 303 | 647 | 1140 | 98 | 61 | 901 | 2200 |
| d | 313 | .. | .. | 415 | 728 | 540 | 381 | 434 | 645 | 2000 |
| e | 306 | .. | .. | 275 | 581 | 1240 | 52 | 58 | 842 | 2192 |

TABLE 3

*Bent ♀ × Dichaete ♂ (carrying translocations).*

| TRANSLOCATION | *D* | + | $Db_l$ | $b_l$ | TOTAL |
|---|---|---|---|---|---|
| a | 286 | — | — | 245 | 531 |
| b | 320 | — | — | 314 | 634 |
| c | 250 | — | — | 285 | 535 |
| d | 209 | — | — | 251 | 460 |
| e | 261 | 2(?) | — | 249 | 512 |

If *D* females carrying a translocation are crossed to $e_y$ males, the progeny consists chiefly of *D* and $e_y$ flies, but some wild-type and $De_y$ individuals also appear (table 2). In males the linkage between *D* and $e_y$ is complete, but in the females these genes may be separated from each other by crossing over. In other words, in the translocations the genes *D* and $e_y$ behave as if they were localized in the same chromosome.

The frequency of crossing over between the loci occupied by *D* and $e_y$ is very different in different translocations. In a- and in b-translocations only 0.2 percent of crossing over occurs between *D* and $e_y$; in c- and e-translocations the frequency of crossing over is 7.2 percent and 5.0 percent respectively; in d-translocation a frequency as high as 40.7 percent was observed.

Two hypotheses explaining the behavior of *D* and $e_y$ in the translocations may be suggested. The first hypothesis is that one of the fourth chromosomes of the treated male carrying the normal allelomorph of $e_y$ has become permanently attached to the *D*-carrying third chromosome. Hence, from such a male all the offspring receiving the *D*-carrying third chromosome receive at the same time the fourth chromosome containing the normal allelomorph of $e_y$, and consequently are Dichaete non-eyeless in appearance. However, in females carrying translocations, *D* and $e_y$ may be separated by crossing over; hence, wild-type and *D* $e_y$ individuals appear along with *D* and $e_y$ in the progeny. The frequency of the crossing over between *D* and $e_y$ depends upon the distance between the locus of *D* and the point of attachment of the fourth chromosome to the third chromosome. Since the frequency of this crossing over is found to be very diverse in the five translocations studied, the fourth chromosome may be assumed to be attached to different parts of the third chromosome.

The second hypothesis assumes that a broken off section of the D-carrying third chromosome from the treated male is attached to the fourth chromosome carrying the normal allelomorph of $e_y$. In other words, the third chromosome was broken into two fragments, and one of these fragments became attached to the fourth chromosome. On this hypothesis males carrying a translocation produce two kinds of gametes in equal numbers (see figure 1). One kind of gametes contains both fragments of the third chromosome, one fragment being attached to the fourth chromosome. These gametes carry, therefore, the gene *D* and the normal allelomorph of $e_y$. The other kind of gametes contains the normal (that is, the unbroken) third chromosome and the free fourth chromosome. These gametes carry $e_y$ but do not carry *D*. If such a male is crossed to a female homozygous for $e_y$ but free from the translocation, only Dichaete non-eyeless and eyeless non-Dichaete individuals are produced (see figure 1). On the other hand, in females carrying a translocation both fragments of the third chromosome involved in the translocation may conjugate with the unbroken third chromosome. If crossing over occurs after such a synapsis, *D* and the normal allelomorph of $e_y$ may be separated from each other. Consequently, *D* $e_y$ and wild-type individuals may appear in the progeny of a female carrying a translocation.

The two hypotheses just outlined are rather similar in nature. Both of them involve the assumption that in the translocations a union between the third and the fourth chromosomes occurred. According to both of them the frequency of crossing over between *D* and $e_y$ indicates the distance between the locus of *D* and the locus of the attachment of the fourth chromo-

some. The only essential difference between these two hypotheses is that the first of them postulates the attachment of the fourth chromosome to the unbroken third chromosome, while the second hypothesis postulates a breakage of the third chromosome and an attachment of the fourth chromosome to one of the resulting fragments of the third. This difference is, however, important from the standpoint of terminology. If the fourth chromosome is attached to the unbroken third, the phenomenon should be

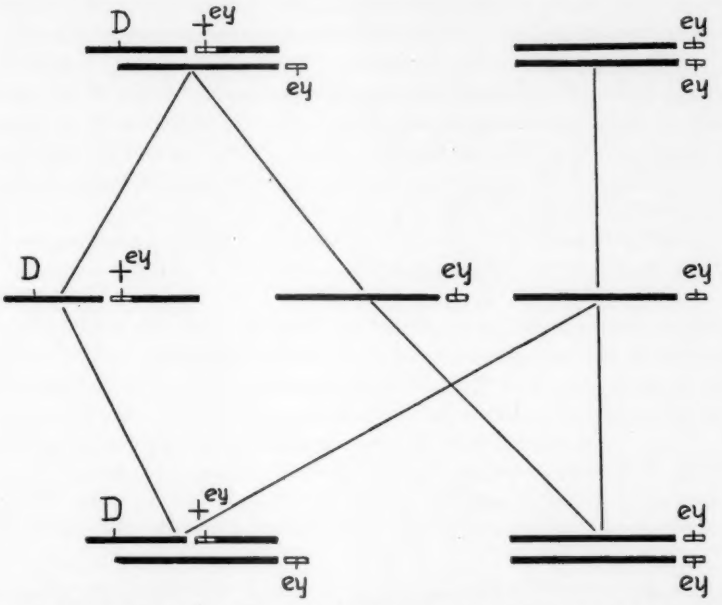



FIGURE 1.—Scheme of a mating of a fly heterozygous for a translocation to a fly free from translocations. Third chromosome—black; fourth chromosome—white. *D*—Dichaete; $e_y$—eyeless. Only gametes giving rise to viable zygotes are represented in this figure.

termed not a translocation but a compounding of chromosomes (see BRIDGES 1923). However, if a fragment of the third chromosome is attached to the fourth, we are dealing with a translocation of a section of the third chromosome on to the fourth.

The cytological investigation is, obviously, the most direct way to the solution of these alternatives. However, before presenting the results of the cytological investigation of the translocations, their genetical behavior may be examined in fuller detail.

## BEHAVIOR OF THE GENES OF THE FOURTH LINKAGE GROUP IN THE TRANSLOCATIONS

The fourth chromosome of *Drosophila melanogaster* is much smaller in size than the other chromosomes of this species. In accordance with this smallness, the number of genes localized in the fourth chromosome is also much less than the number of the genes in other chromosomes. Furthermore, no (or very little) crossing over takes place between the known genes of the fourth chromosome (unpublished data of BRIDGES). It seems *a priori* very probable that in translocations the behavior of all the fourth chromosome genes is identical with the behavior of $e_y$ which has been discussed above. Both hypotheses advanced in the preceding section assume that in all the translocations the whole fourth chromosome rather than a fragment of it is attached to the third chromosome. The extreme smallness and the absence of crossing over in the fourth chromosome make the contrary supposition improbable.

To test the validity of this point, *D* males carrying translocations were crossed to females homozygous for bent. Bent ($b_t$) is a fourth-chromosome recessive gene producing a shortening of the legs and a spreading of the wings of flies. From the $F_1$ of this cross *D* males were selected and backcrossed to homozygous $b_t$ females. In the next generation only *D* and $b_t$ (no wild-type and no *D* $b_t$) individuals appeared (table 3). The two wild-type flies shown in table 3 were probably genotypically $b_t$; their appearance is due to the fact that $b_t$ some times overlaps wild type phenotypically. When *D* females from the $F_1$ were crossed to $b_t$ males, some wild-type and *D* $b_t$ individuals appeared along with *D* and $b_t$ individuals. The behavior of $b_t$ in translocations is, therefore, identical with behavior of $e_y$.

The presence of a linkage between *D* and the fourth-chromosome dominant gene Minute-IV in translocations was proved by a similar method. Dichaete males carrying translocations were crossed to females heterozygous for Minute-IV (Minute-IV is lethal when homozygous). In the $F_1$ generation Dichaete Minute-IV males were selected and crossed to wild-type females. Only *D* and Minute-IV (no wild type and no Dichaete Minute-IV) were produced in the progeny.

Although the two remaining genes located in the fourth chromosome (namely shaven and rotated abdomen) were not tested in the translocations, there can be no doubt that their behavior is identical with the behavior of $e_y$, $b_t$ and Minute-IV.

## CROSSING OVER IN THE THIRD CHROMOSOME INVOLVED IN THE TRANSLOCATIONS

A series of experiments was necessary to determine the exact loci in the third chromosome at which the fourth chromosome is attached in the different translocations. The exact determination of these loci is especially important, since, according to the second hypothesis outlined above, they may coincide (and as it will be proved later do coincide) with the breaking-points of the third chromosome in the translocations.

Dichaete males carrying translocations were crossed to females homozygous for $e_y$ and homozygous for the third-chromosome recessive genes roughoid ($r_u$-rough eye-surface), hairy ($h$-hairs along the wing-veins), thread ($t_h$-unbranched aristae), scarlet ($s_t$-bright red eye-color), curled ($c_u$-wings curved upwards), stripe ($s_r$-dark longitudinal stripe on the thorax), sooty ($e^s$-dark body-color) and claret ($c_a$-purplish pink eye-color). Dichaete females were selected in the $F_1$ generation of this cross and backcrossed to $e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ males. The results obtained in the next generation are presented in tables 11-15 (appendix). (Flies homozygous for the allelomorph of $e_y$ used, namely $e_y^2$, rarely have both eyes completely absent. In flies having no eyes the classification in respect to $r_u$, $s_t$ and $c_a$ is, of course, impossible. Such flies were disregarded and are not included in the counts presented in tables 11-15. The frequency of homozygous $e_y^2$ flies having no eyes is, however, so low that their omission can not influence appreciably the crossing over values obtained.)

The frequency of crossing over in an interval between two given genes depends to a certain extent upon the modifying genes present in the stock used. Therefore, it is not desirable to compare the frequency of crossing over in the third chromosome involved in the translocations directly with the frequency of crossing over in the normal third chromosome indicated by the standard map of this chromosome (for the standard map see figure 2, also MORGAN, BRIDGES, STURTEVANT 1925, p. 92). With the object of having a better standard of comparison a special control experiment was made. Dichaete males from the same stock of *D* flies from which the *D* males treated by X-rays were obtained (see above) were crossed to homozygous $e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ females from the same stock which was used for the study of crossing over in the translocations. In the $F_1$ generation *D* females were selected and backcrossed to $e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ males. The results obtained in the next generation are recorded in table 16 (appendix).

The crossing over values were calculated from the data shown in tables 11-16. The calculated crossing over values are presented in table 4. As

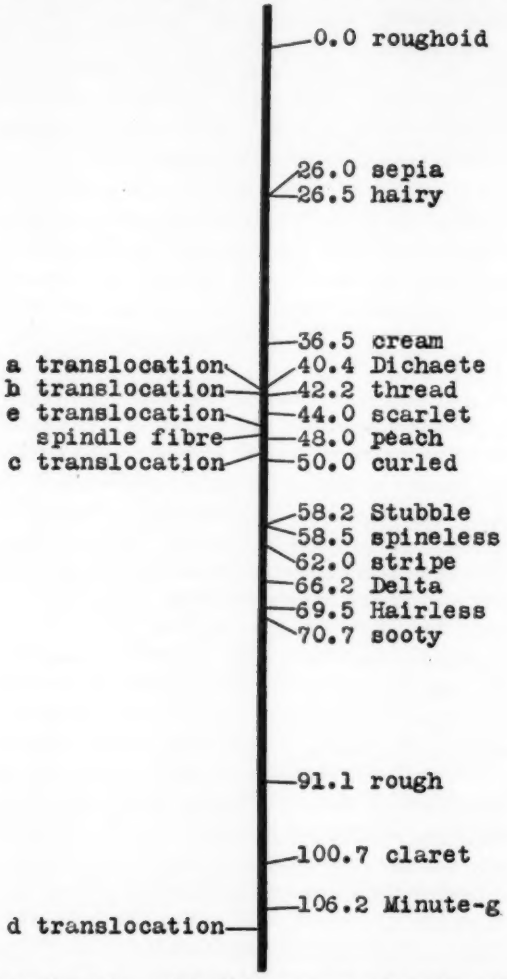



FIGURE 2.—Genetical map of the third chromosome of *Drosophila melanogaster.*

seen from table 4, the point of attachment of the fourth chromosome to the third (designated in table 4 by +) is not alike in the different translocations.

In a-translocation this point is located between the genes *D* and $t_h$. In b-translocation the point of attachment is nearly the same as in a-translocation but in b-translocation it seems to coincide with the locus of the gene $t_h$. In c-translocation and in e-translocation the fourth chromosome is attached near the middle of the length of the third chromosome, namely in the $s_t$-$c_u$ interval. However, in e-translocation the point of attachment is located nearer to $s_t$ than to $c_u$, whereas in c-translocation it is nearer to $c_u$ than to $s_t$. Finally, in d-translocation the fourth chromosome is attached near the extreme right-hand end of the third chromosome, 5.1 units to the right of the gene $c_a$.

TABLE 4

*Crossing over values for the intervals studied in the third chromosome.*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Translocation a. | | | | | |
| Interval | $r_u$-$h$ | $h$-$D$ | $D$-+ | +-$t_h$ | $t_h$-$s_t$ | $s_t$-$c_u$ | $c_u$-$s_r$ | $s_r$-$e^a$ | $e^a$-$c_a$ |
| Value | 17.2 | 2.9 | .05 | .1 | .2 | 6.6 | 16.6 | 11.7 | 31.0 |
| | | | | Translocation b. | | | | | |
| Interval | $r_u$-$h$ | $h$-$D$ | $D$-+-$t_h$ | $t_h$-$s_t$ | $s_t$-$c_u$ | $c_u$-$s_r$ | $s_r$-$e^a$ | $e^a$-$c_a$ | |
| Value | 21.2 | 5.9 | .2 | .3 | 4.6 | 17.1 | 10.0 | 31.0 | |
| | | | | Translocation c. | | | | | |
| Interval | $r_u$-$h$ | $h$-$D$ | $D$-$t_h$ | $t_h$-$s_t$ | $s_t$-+ | +-$c_u$ | $c_u$-$s_r$ | $s_r$-$e^a$ | $e^a$-$c_a$ |
| Value | 26.9 | 15.4 | 1.5 | .9 | 3.8 | .7 | 3.7 | 7.5 | 28.9 |
| | | | | Translocation d. | | | | | |
| Interval | $r_u$-$h$ | $h$-$D$ | $D$-$t_h$ | $t_h$-$s_t$ | $s_t$-$c_u$ | $c_u$-$s_r$ | $s_r$-$e^a$ | $e^a$-$c_a$ | $c_a$-+ |
| Value | 29.1 | 14.8 | 1.1 | .9 | 5.9 | 12.6 | 8.9 | 19.2 | 5.1 |
| | | | | Translocation e. | | | | | |
| Interval | $r_u$-$h$ | $h$-$D$ | $D$-$t_h$ | $t_h$-$s_t$ | $s_t$-+ | +-$c_u$ | $c_u$-$s_r$ | $s_r$-$e^a$ | $e^a$-$c_a$ |
| Value | 19.5 | 11.6 | 1.1 | .5 | 1.5 | 3.6 | 15.6 | 11.6 | 31.0 |
| | | | | Control | | | | | |
| Interval | $r_u$-$h$ | $h$-$D$ | $D$-$t_h$ | $t_h$-$s_t$ | $s_t$-$c_u$ | $c_u$-$s_r$ | $s_r$-$e^a$ | $e^a$-$c_a$ | |
| Value | 23.7 | 13.7 | 1.1 | .8 | 8.0 | 15.4 | 10.3 | 31.0 | |

Comparison of the crossing over values for the different intervals of the third chromosome observed in the translocation and in the control experiment reveals the presence of consistent differences between them (see table 5). In a, b- and in e-translocations the frequency of crossing over is markedly decreased to the left of the $s_t$-$c_u$ interval but slightly increased to the right of this interval. On the other hand, in c- and d-translocations crossing over is decreased to the right of the $s_t$-$c_u$ interval but increased to the left of it.

These regularities may be described also as follows. A point located approximately in the middle of the $s_t$-$c_u$ interval divides the third chromosome into two limbs. If the fourth chromosome is attached to the third

chromosome to the left of this point (as is the case in a-, b- and e-translocations), the frequency of crossing over is decreased in the left limb but increased in the right limb of the third chromosome. If, however, the point of the attachment of the fourth chromosome lies to the right of the point mentioned above (as is the case in c- and d-translocations), the frequency of crossing over is decreased in the right limb of the third chromsome but increased in the left limb (see figure 2). (Following the practice established in the Drosophila literature, the end of the third chromosome at which the gene $c_a$ is located is designated in this discussion as the right-hand end, and the end at which the gene $r_u$ is located as the left-hand end of this chromosome.)

TABLE 5

*Differences between the crossing-over values in the translocations and the corresponding values observed in the control experiment.*

| INTERVAL | | TRANSLOCATION | | | | |
|---|---|---|---|---|---|---|
| | | a | b | c | d | e |
| Left of the spindle fibre | $r_u$-$h$ | −6.5 | −2.5 | +3.2 | +5.4 | −4.2 |
| | $h$-$D$ | −10.8 | −7.8 | +1.7 | +1.1 | −2.1 |
| | $D$-$l_h$ | −.95 | −.9 | +.4 | 0.0 | 0.0 |
| | $l_h$-$s_t$ | −.6 | −.5 | +.1 | +.1 | −.3 |
| Spindle fiber | $s_t$-$c_u$ | −1.4 | −3.4 | −3.5 | −2.1 | −2.9 |
| Right of the spindle fibre | $c_u$-$s_r$ | +1.2 | +1.7 | −11.7 | −2.8 | +.2 |
| | $s_r$-$e^s$ | +1.4 | −.3 | −2.8 | −1.4 | +1.3 |
| | $e^s$-$c_a$ | 0.0 | 0.0 | −2.1 | −11.8 | 0.0 |

The strongest reduction of the frequency of crossing over is observed in the intervals adjacent to the locus of the attachment of the fourth chromosome. Nevertheless, some reduction of crossing over takes place in all the intervals of the limb of the third chromosome in which the point of attachment of the fourth chromosome lies. For instance, in d-translocation the greatest decrease of the frequency of crossing over is observed in the $e^s$-$c_a$ interval, while in the $c_u$-$s_r$ interval the reduction is comparatively slight. In d-translocation the point of attachment of the fourth chromosome lies near the $e^s$-$c_a$ interval. On the contrary, in c-translocation the frequency of crossing over is strongly decreased in the $c_u$-$s_r$ interval, while in the $e^s$-$c_a$ interval it is but slightly affected. In c-translocation the fourth chromosome is attached to the left of $c_u$. It is significant, however, that if the attachment of the fourth chromosome is near to the point dividing the third chromosome in two limbs (for example, the point lying half way between $s_t$ and

$c_u$) the decrease of the crossing over frequency does not affect the adjacent intervals of both limbs (see c- and e-translocations). This fact suggests that the point dividing the two limbs of the chromosome plays some especially important role in the distribution of the crossing over along the third chromosome.

Most of the differences between the crossing over values for the respective intervals observed in the translocations and in the control experiment are statistically significant. Furthermore, the consistency of the results obtained suggests that these differences are significant even in the cases where the value of the difference in question is below the limit of statistical certainty.

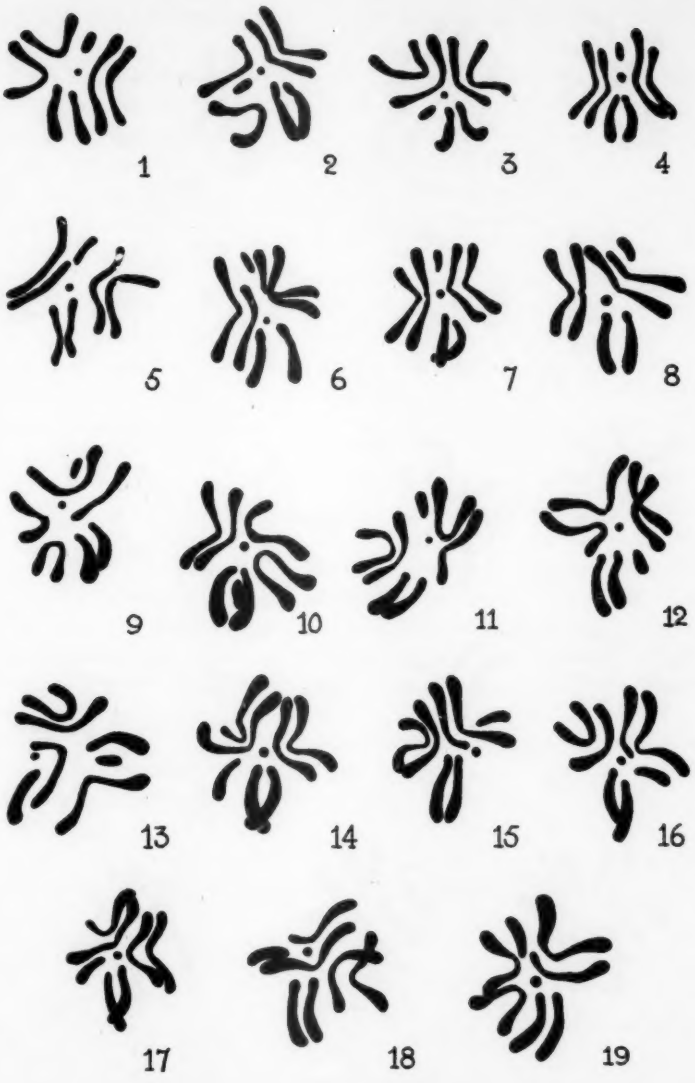
It might be supposed that differential viability of the various classes of crossover individuals is responsible for the alteration of the crossing over frequencies in the translocations. Since the point of attachment of the fourth chromosome to the third is not the same in different translocations the distribution of $e_y$ among the crossovers is not alike in different cases. The gene $e_y$ in homozygous condition causes some decrease of the viability of the flies. However, the difference between the crossing over values found in the different translocations can not be accounted for by the influence of $e_y$ on the viability of the flies. Indeed in both c-and e-translocations the locus of the fourth chromosome attachment lies in the $s_t$ - $c_u$ interval. The distribution of $e_y$ among the crossing-over individuals is, therefore, alike in c- and e-translocations. Nevertheless, in c-translocation there is observed a decrease of the frequency of crossing over in the right limb and an increase in the left limb of the chromosome, while in e-translocation a decrease takes place in the left limb and an increase in the right limb.

The third chromosome of *Drosophila melanogaster*, as seen in the cytological preparations, is V-shaped, and the point of attachment of the spindle fibre to the body of the chromosome lies at the middle of its length, dividing the chromosome into two limbs of equal size. It is very probable that the decrease of the frequency of crossing over in one part of the chromosome and the increase of its frequency in the other part of the same chromosome is due to the location of the point of the attachment of the fourth in relation to the spindle fibre. If the fourth chromosome is attached to the left of the locus of the attachment of the spindle fibre (in a-, b- and e-translocations) crossing over is reduced in the left limb of the chromosome and increased in the right limb. If, however, the fourth chromosome is attached to the right of the spindle fibre (as in c-and d-translocations), the crossing over frequency is decreased in the right limb but increased in

## Explanation of Plates 1 and 2

The drawings represent chromosomes (oögonial plates) of the females carrying translocations in heterozygous (figures 1–33) or in homozygous (figures 34–38) condition. All the drawsings are done at the level of the work-table with the aid of camera lucida, under the Zeiss immers. obj. 1.5 and comp. oc. 30. The scale given in plate 2 below, common to all the drawings, represents 10 micra. As far as possible the figures were placed so that the X-chromosomes occupy the lower part of each figure.

Plate 1, figures 1–6 represent a-translocation; figures 7–13 b-translocation; figures 14–19 c-translocation (heterozygous); plate 2, figures 20–24 d-translocation; figures 25–33 e-translocation; figures 34–38 homozygous c-translocation.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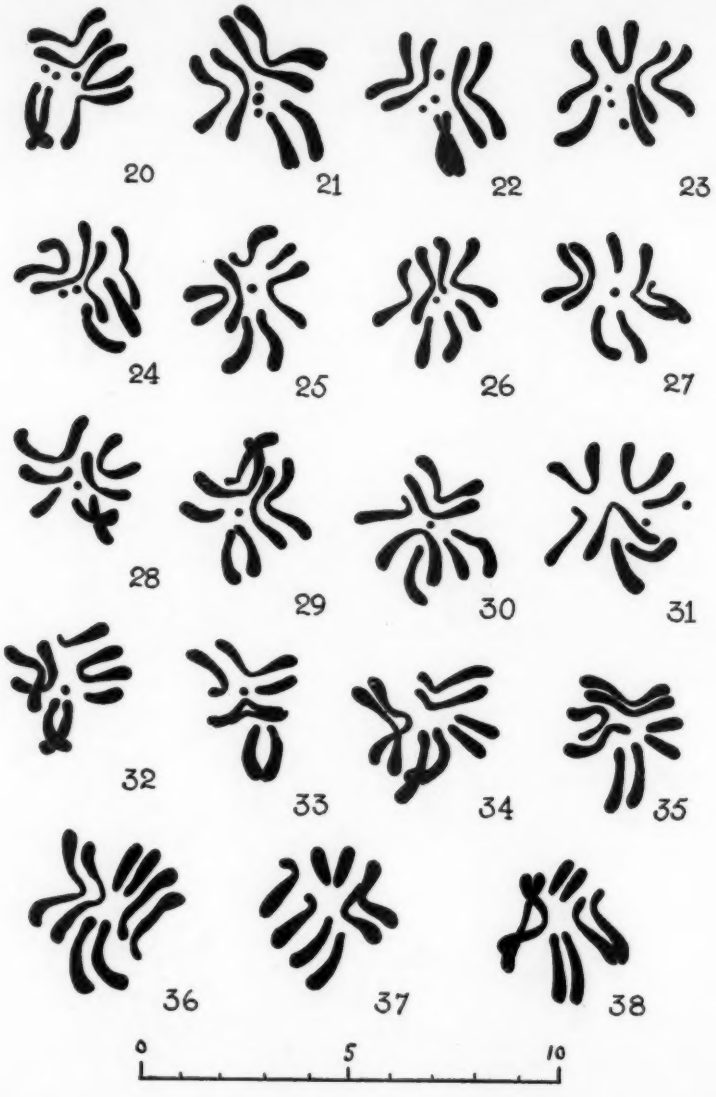
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the left limb of the chromosome. It is a plausible assumption that the point lying half way between $s_t$ and $c_u$ which divides the third chromosome in two "genetical limbs" (see above) is in reality the point of spindle-fibre attachment. The location of this point approximately in the middle of the length of the map of the third chromosome (see figure 2) is in good agreement with such an assumption, since the attachment of the spindle fibre is located cytologically also at the middle of the length of the chromosome.

It must be emphasized that the location of the point of spindle-fibre attachment between the loci of $s_t$ and $c_u$ was already probable on the basis of the data secured by quite independent methods. BRIDGES and MORGAN (1923) on the basis of the study of the distribution of double crossing over in the third chromosome came to the conclusion that the point of the spindle-fibre attachment lies between the loci of $s_t$ and peach (the locus of peach is between $s_t$ and $c_u$). STURTEVANT came to the same conclusion on the basis of the study of inverted sections (STURTEVANT and PLUNKETT 1926, also unpublished data). Recently REDFIELD (unpublished) obtained further evidence pointing in the same direction, from investigations of the crossing over frequency in the third chromosome of triploid females.

It has been shown above that in c-translocation the frequency of crossing over is decreased in the right limb and increased in the left limb of the third chromosome, while in e-translocation the conditions are reversed. It follows that the point of spindle-fibre attachment lies between the loci of attachment of the fourth chromosome in c- and in e-translocation. It is known, furthermore, that in both c- and e-translocations the fourth chromosome is attached to the third between the genes $s_t$ and $c_u$. Therefore it becomes especially important to determine the location of the fourth-chromosome attachment in c- and e-translocations in respect to the gene peach ($p$-eye color) which lies within the $s_t$ -$c_u$ interval.

TABLE 6

*Crossing over in* $\frac{e_y \quad D}{s_t \; p \; c_u} \; ♀ \times e_y \, D \, s_t \, p \, c_u \, ♂$.

Translocation c.

| Interval | $D$-$s_t$ | $s_t$-$p$ | $p$-+ | +-$c_u$ | Total flies examined |
|---|---|---|---|---|---|
| Value | 2.2 | 3.4 | .07 | .2 | 1481 |

Translocation e.

| Interval | $D$-$s_t$ | $s_t$-+ | +-$p$ | $p$-$c_u$ | Total flies examined |
|---|---|---|---|---|---|
| Value | .8 | 1.2 | 1.1 | 2.4 | 1288 |

Dichaete males carrying c- and e-translocations respectively were crossed to females homozygous for $e_y$, $s_t$, $p$ and $c_u$. In the $F_1$ generation $D$ females were selected and backcrossed to $e_y \, s_t \, p \, c_u$ males. The results

obtained in the next generation are summarized in table 6. As seen from table 6, in e-translocation the locus of attachment of the fourth chromosome lies in the $s_t$-$p$ interval while in c-translocation it lies in the $p$-$c_u$ interval. The frequency of crossing over in the $D$-$s_t$ and the $s_t$-$p$ intervals is considerably lower in e-translocation than in c-translocation. On the contrary, the frequency of crossing over in the $p$-$c_u$ interval is lower in c-translocation than in e-translocation.

It may be concluded on the basis of this evidence that the point of the spindle-fibre attachment is located near the gene peach, probably slightly to the left of it. In other words, the spindle fibre is attached to the third chromosome between the loci 46 and 48 of the standard map (see figure 2).

### CYTOLOGICAL CHANGES PRODUCED BY THE TRANSLOCATIONS

It has been pointed out above that the genetical behavior of the translocations may be explained on either of two assumptions. First, the fourth chromosome may have become permanently attached to the third chromosome. Second, a section of the third chromosome may have become broken off and attached to the fourth chromosome. The observed alterations of the frequency of crossing over in the the third chromosome might be produced, judging *a priori*, in either case, In other words, the genetical data so far presented give no evidence as to which of these two assumptions is correct. This, however, may be easily decided by the cytological investigation of the translocations. If the fourth chromosome in the translocations is attached to the unbroken third, only one instead of two fourth chromosomes must be present in the chromosomal plates. Except this, the chromosomes must look normal, since the point of the attachment of the fourth chromosome will be, probably, indistinguishable because of the extreme smallness of this chromosome, However, if a section of the third chromosome is broken off and attached to the fourth chromosome, one of the cytologically visible third chromosomes must be shortened, and one of the fourth chromosomes must be increased in size because of the addition of the material from the third chromosome.

Ovaries of freshly hatched females or of mature pupae were dissected and fixed successively in strong Flemming's fluid (1 hour) and in 1 percent solution of chromic acid (20 hours). Not less than 7 sufficiently clear chromosome plates were obtained for each of the five translocations. Most of them are presented in plates 1 and 2 (see also figures in DOBZHANSKY 1929a).

It is most convenient to begin the presentation of the results of the cytological investigation of the translocations with a description of the features in common to a-, b-, c- and e-translocations (plate 1, figures 1–19,

plate 2, figures 25–33). The overwhelming majority of the chromosomal plates studied in these translocations (52 plates out of 54 studied) contain only one free fourth chromosome of normal size. This fact is important, since it may be considered as a cytological proof of our basic assumption, namely that the observed linkage between the genes belonging to the third and to the fourth linkage groups is due to translocations involving these chromosomes. Individuals having only one fourth chromosome are known in *Drosophila melanogaster*, but they always have a complex of external peculiarities called "Haplo-IV" or "Diminished" (BRIDGES 1921). However, flies carrying translocations never possess the characteristics of Haplo-IV. This fact may be explained only on the assumption that the flies carrying translocations have in reality two fourth chromosomes, one free, and the other attached to the third chromosome or to a fragment of the latter. The few cases in which two free fourth chromosomes are found in the translocations are probably due to non-disjunction of the fourth chromosomes. In these cases the individuals have in reality three fourth chromosomes, two of them being free and one attached to the third chromosome. The plausibility of this explanation will be examined below.

The pair of X-chromosomes and the smaller pair of the V-shaped autosomes appear to be perfectly normal in all plates. However, the larger pair of the V-shaped autosomes consists of two partners distinctly unequal in length, although showing somatic pairing with each other in the majority of the plates studied. The longer partner is symmetrical, that is, it has both limbs equally long. The equality of the length of the limbs of the longer partner is especially clearly seen in the cases in which the median constriction of the chromosome is clearly pronounced (see plate 1, figures 6, 7, 8, 12, 16, 17, 18, plate 2, figures 27, 31). On the other hand, the shorter partner is asymmetrical, that is, it has one of the limbs markedly shorter than the other. The inequality of the two limbs of the asymmetrical partner is manifest in all plates studied, but it is especially striking in the case where the median constriction is pronounced (see plate 1, figures 3, 7, 10, 14, plate 2, figures 27, 29, 33).

Since the V-shaped autosomes in the chromosomal plates of the normal flies have both limbs equal in length, it is possible to conclude that the asymmetrical autosomes just described are the third chromosomes involved in the translocations.

The asymmetrical chromosome is the member of the larger pair of the V-shaped autosomes. The difference in length between the larger and the smaller pair of the V-shaped autosomes is clear enough in the majority of

the chromosomal plates studied. Among all the plates presented here perhaps only those in figure 5 (plate 1) and in figure 23 (plate 2) may be uncertain as to which of the two pairs of the V-shaped autosomes is the larger and which the smaller. Therefore, it is possible to conclude that the larger pair of the-V shaped autosomes of *Drosophila melanogaster* carries the third-chromosomal linkage group of genes, whereas the smaller pair carries the second-chromosome linkage group. In what follows we shall call the larger pair of the V-shaped autosomes the third chromosome, and the smaller pair the second chromosome.

This identification of the third and the second chromosomes is in disagreement with the identification given by BRIDGES (MORGAN, STURTEVANT, BRIDGES 1928), MULLER and PAINTER (1929) and PAINTER and MULLER (1929). Indeed, the authors mentioned identified the smaller pair of the V-shaped autosomes in *Drosophila melanogaster* as being the carrier of the third-chromosome linkage group, and the larger pair as the carrier of the second-chromosome linkage group of genes. The conclusions of BRIDGES were based on the cytological study of "Pale" translocation in which case only a very small fragment of the second chromosome has been broken off and attached to the third chromosome. The cytological differences observed were so slight, that no decisive conclusion could be drawn. In fact, BRIDGES himself previously identified the larger pair as the third chromosome and the smaller pair as the second chromosome (MORGAN, BRIDGES, STURTEVANT 1925, p. 177) on the basis of the study of the same "Pale" translocation. Doctor C. B. BRIDGES has seen my preparations and agrees with my interpretation. MULLER's and PAINTER's material is quite dependable, since the translocations described by these authors involve large sections of the second and third chromosomes. But, as far as I can see, at least some of PAINTER's own figures representing the cytological conditions in the translocations involving the second or the third chromosomes show the third chromosome longer than the second (see MULLER and PAINTER 1929, figures 1, 3, 5, 6, PAINTER and MULLER 1929, figure 29). As far as the length of the chromosomes is concerned there remains no doubt that the third chromosome is longer than the second.

Both ends of the normal V-shaped autosomes are thicker than the middle part of the same chromosomes. It is worth notice that the shorter limb of the asymmetrical third chromosome found in the translocations is always less thickened at the end than the longer limb of the same chromosome. The longer limb of the asymmetrical third chromosome is equal

in length and shape to either limb of the normal third chromosome present in the same plate.

Obviously a loss of some material normally located in the third chromosome is responsible for the shortening of one of the limbs of the asymmetrical third chromosome. This material is undoubtedly to be looked for in the small, rod-shaped, unpaired chromosome clearly visible in all the chromosomal plates in the translocations but not present at all in the normal chromosomal plate of *Drosophila melanogaster*. The length of the rod-shaped chromosome plus the length of the asymmetrical third chromosome is about equal to the length of the normal third chromosome present in the same plate. One of the ends of the new rod-shaped chromosome (that directed toward the periphery of the chromosomal plate) is in most cases thicker than the other end of the same chromosome (directed toward the center of the plate). The thickness of the thicker end of the rod-shaped chromosome is approximately equal to (or a little smaller than) the thickness of the ends of the normal third chromosome.

The interpretation of the chromosomal relations found in a-, b-, c- and e-translocations is obvious. In each of these translocations a third chromosome is broken into two fragments of unequal length. The shorter of these fragments is attached to a fourth chromosome. The resulting compound-chromosome (a fragment of the third chromosome plus the fourth chromosome) is seen cytologically as the unpaired rod-shaped chromosome just described. The longer of the fragments is represented by the asymmetrical third chromosome, having one limb markedly shorter than the other limb. The end of the shorter limb of the asymmetrical third chromosome corresponds to the point at which the third chromosome has been broken. Finally, since one of the fourth chromosomes is included in the unpaired rod-shaped chromosome, only, one free fourth chromosome is seen in most of the plates. In other words, the second of the two hypotheses suggested for the explanation of the genetical behavior of the translocations is confirmed by the cytological data.

According to the genetical data presented in the preceding section, the points at which the breakage of the third chromosome occured in a-, b,- c- and e-translocations are different (see figure 2). If the genetical map represents the real arrangement of the genes in the third chromosome, the broken off sections are of different lengths in different translocations. Consequently, the size of the asymmetrical third chromosome as well as the size of the unpaired rod-shaped chromosome must be differentiated in the different translocations studied.

In a- and b-translocations the third chromosome is broken between the loci of the genes *D* and $t_h$; in b-translocation the breakage occurred very slightly nearer to the middle of the chromosome than in a-translocation. The cytological conditions found in a- and b-translocations are nearly alike (a-translocation plate 1, figures 1–6, b-translocation plate 1, figures 7–13). The length of the unpaired rod-shaped chromosome equals about one-third the length of the limb of the normal third chromosome. The asymmetrical third chromosome has one limb shorter than the other by approximately one-third of its length. In b-translocation the rod-shaped chromosome seems to be very slightly longer than in a-translocation, and correspondingly the asymmetrical third chromosome is slightly shorter in b-translocation than in a-translocation. According to the genetical data the rod-shaped chromosome in these cases must carry the genes from *D* to the left end of the third chromosome (including the gene $r_u$), and the asymmetrical third chromosome must carry the genes from $t_h$ to right end of the third chromosome (including $c_a$).

In e-translocation the third chromosome is broken between the locus of $s_t$ and the hypothetical locus of the spindle-fibre attachment. Consequently, the rod-shaped chromosome in this case contains the genes from $s_t$ to $r_u$, and therefore must be longer than the rod-shaped chromosome in a- and b-translocations (compare figure 2). The asymmetrical third chromosome in e-translocation carries the genes from $p$ to $c_a$, and, therefore, its shorter limb must be considerably shorter than in a- and b-translocations. This expectation is confirmed by the cytological investigation of e-translocation (plate 2, figures 25–33). The length of the rod-shaped chromosome equals five-sixths or four-fifths the length of the limb of the normal third chromosome. The asymmetrical third chromosome has one limb very short. It is clearly visible, in cases in which the median constriction is pronounced, that the third chromosome is broken very near its middle, that is, near the point of attachment of the spindle fibre (see figures 27, 29, 33).

In c-translocation the third chromosome is broken between the genes $p$ and $c_u$, that is to the other side of the spindle-fibre attachment as compared with e-translocation. The rod-shaped chromosome in this case contains the genes from $c_u$ to $c_a$, and the asymmetrical third chromosome carries the genes from $p$ to $r_u$. Cytologically (plate 1, figures 14–19) the conditions found in c-translocation are similar to those in e-translocation, but here the rod-shaped chromosome is slightly shorter and the asymmetrical third chromosome slightly longer than in e-translocation. This fact indicates

that the locus of attachment of the spindle fibre is cytologically nearer to the point at which the third chromosome is broken in e-translocation than to the point of breakage in c-translocation.

The chromosomes in d-translocation are at first glance like those of a normal fly. Both pairs of V-shaped autosomes as well as the X-chromosomes appear to be normal. However, one of the fourth chromosomes is clearly increased in size, being roughly from 1½ to twice as large as the other fourth chromosomes present in the same plates. Only in a single plate (figure 24) is the increase in size of one of the fourth chromosomes not distinct. There are present three free fourth chromosomes in most plates in d-translocation, indicating the high frequency of non-disjunction of the fourth chromosomes in this translocation.

The increase in size of one of the fourth chromosomes indicates that the nature of d-translocation is the same as the nature of the other translocations studied, namely that here also a section of the third chromosome is broken off and attached to the fourth chromosome. However, in D-translocation the section of the third chromosome removed from its normal location is so small that its loss is not noticeable in the cytologically visible third chromosome because the third chromosome itself is relatively very large. Nevertheless, the addition of this section to the fourth chromosome is visible cytologically because of the extreme smallness of the latter chromosome.

This explanation agrees very nicely with the genetical data on d-translocation. According to the genetical data the point of breakage of the third chromosome in d-translocation is located 5.1 units to the right of $c_a$, that is, near the extreme right-hand end of the third chromosome. The map of the third chromosome shows the gene Minute-g located 5.5 units to the right of $c_a$ (figure 2). However, in d-translocation the break in the third chromosome is probably to the right of Minute-g, for the crossing over value for the $e^s$-$c_a$ interval is 19.2 percent in d-translocation instead of 31.0 units observed in the control. Since in d-translocation the frequency of crossing over is considerably decreased in the right limb of the chromosome, the claret - Minute-g interval is probably considerably shorter than 5.5 units. Correspondingly, the point of the breakage of the third chromosome is located to the right of Minute-g, in the unknown part of the extreme right-hand end of the third chromosome. The size of the section of the third chromosome which has been broken off and attached to the fourth chromosome must be very small in d-translocation.

It is a remarkable fact that in all five cases of translocations studied there is observed a breakage of the third chromosome and an attachment

of the broken off section to the fourth chromosome. In not a single case is the fourth chromosome attached to the unbroken third chromosome. Furthermore, the fragment of the third chromosome attached to the fourth never includes the region of the third chromosome in which the spindle-fibre attachment lies. On the contrary, the fragment of the third chromosome remaining free always includes the region of the spindle-fibre attachment.

These relations suggest the possible manner of origin of the translocation described. The chromosomes treated by X-rays are likely to stick together because of the increase of ionization produced by the rays. An attachment of the fourth chromosome to the third may occur. However, the resulting compound has two spindle fibres (one contributed by the third chromosome and the other by the fourth chromosome). The two spindle fibres may pull the compound chromosome to opposite poles of the spindle during mitosis, producing the breakage of the chromosome, or else the rate of contraction of the two spindle fibres may be different, in which event the breakage of the compound-chromosome also may take place. Both resulting fragments have one and only one spindle fibre, and therefore behave during mitosis like normal chromosomes. It seems very probable that a fragment of a chromosome can be normally transmitted from one cell-generation to the other only when it has its own spindle fibre, or acquires a spindle fibre by an attachment to another chromosome or to a fragment of a chromosome possessing a spindle fibre.

The points of breakage of the third chromosome observed in the translocations do not show a tendency to coincide with the constrictions in the chromosome described by BRIDGES (1927). According to BRIDGES, the third chromosome of *Drosophila melanogaster* has a strong median constriction at the point of the spindle-fibre attachment, and two other less pronounced constrictions in each of the two limbs. One of the latter constrictions, the submedian, lies at about one-fifth of the limb-length from the median constriction; another, still less pronounced, lies at about one-quarter of the limb-length from the end of the chromosome. None of the observed breakages of the third chromosome coincide with the median constriction. The other two constrictions are so inconspicuous that it is difficult to decide whether in a given case the point of breakage coincides with one of them. However, the breakage point in both c- and e-translocations can not coincide with the submedian constriction. Indeed, in c-translocation the point of breakage of the chromosome is located further from the median constriction than in e-translocation, whereas

the submedian constrictions in both limbs of the chromosome are equidistant from the median constriction. Likewise, the point of breakage either in a- or in b-translocation may be supposed to coincide with the constriction located at one-fourth of the limb-length from the end of the chromosome. However, at least in one of these cases the point of breakage certainly does not coincide with the constriction, since, as the genetical data show, the breakages in a- and b-translocations are not located at the same point. Finally, the breakage observed in d-translocation is located in a region of the third chromosome in which no constrictions are known.

## GENETICAL CONSEQUENCES OF THE BREAKAGE OF THE THIRD CHROMOSOME IN THE TRANSLOCATIONS

In each of the translocations the locus in the third chromosome at which the fourth chromosome is attached was determined by studying the linkage relations of the genes of the third and fourth linkage groups. It has been pointed out above that this method gave no evidence on the question whether the fourth chromosome is attached to the unbroken third, or a section of the third chromosome has been broken off and attached to the fourth chromosome. This question was solved by the cytological investigation of the translocations. Indeed, in each of the five translocations studied the third chromosome was observed to be broken into two fragments and the shorter of these fragments is attached to the fourth chromosome. It is obviously desirable to check the results of the cytological investigation by an *experimentum crucis*, that is, to demonstrate by purely genetical methods the presence of a break of the third chromosome in the translocations.

As shown by the cytological study, one normal (unbroken) third chromosome, one third chromosome broken into two fragments and one free fourth chromosome are present in each of the cells of the flies carrying translocations. Another fourth chromosome is also present in each of the cells, but it is attached to the shorter fragment of the third chromosome, and therefore is not distinguishable cytologically (see figures 1 and 3). Let us consider the distribution of these chromosomes at the reduction during the gametogenesis.

The unbroken third chromosome and the free fourth chromosome might go to one pole of the spindle at the reduction division, and the two fragments of the broken third chromosome might go together with the attached fourth chromosome to the other pole (figures 1 and 3). Gametes 1 and 2 (figure 3) will be formed in this case. However, either of the frag-

ments of the broken third chromosome might go together with the unbroken third chromosome to the same pole of the spindle. As a result of such a distribution of the chromosome at the reduction division some of the gametes formed will carry a complete third chromosome plus a fragment of the other third chromosome (hyperploid gametes, figure 3, gametes 3 and 5). The other gametes will carry only one of the fragments of the third chromosome but will be deficient in respect to the other fragment of the same chromosome (hypoploid gametes, figure 3, gametes 4 and 6).

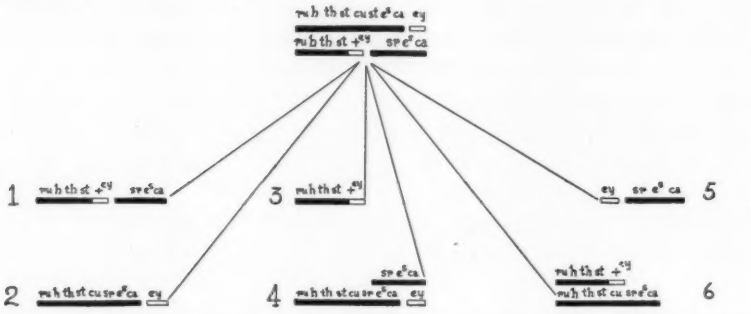


FIGURE 3.—Six kinds of gametes produced by a fly heterozygous for a translocation. Third chromosome—black; fourth chromosome—white.

The gametes 1 and 2 (figure 3) may be called "regular" gametes, whereas gametes 3 to 6 are "non-disjunctional" for one or the other section of the third chromosome. If a fly carrying a translocation is crossed to a fly free from translocation the regular gametes will give rise to zygotes carrying translocation and to normal zygotes respectively (figure 1). The non-disjunctional gametes will give rise to zygotes carrying a duplication or a deficiency for a section of the third chromosome. Flies developing from the latter zygotes must be expected to possess external peculiarities due to the genic unbalance. No such flies have been found in the cultures of any of the translocations here described. Hence, the non-disjunctional gametes are either not produced, or, more probably, the somatic changes in the zygotes, due to the changes in genic balance, are so great that the zygotes are unable to survive.

It is possible to test for the production of the non-disjunctional gametes by a genetical method. If two flies both carrying the same translocation are crossed to each other, gamete 3 from one parent united with gamete 4 from the other parent will give a normal zygote, since these gametes are com-

plementary to each other. Likewise, gamete 5 from one parent united with gamete 6 from the other parent must also give a normal zygote for the same reason. It is easy to make the zygotes produced by the union of the non-disjunctional gametes distinguishable in appearance from the zygotes produced by the union of the regular gametes. For this purpose it is necessary to introduce some recessive mutant genes into the chromosomes involved in the translocations.

Flies carrying translocations and at the same time homozygous in respect to various third-chromosome recessive genes may be easily obtained. In fact, many such flies have been observed during the course of the experiments planned for the purpose of studying the frequency of crossing over in the third chromosome (all the non-eyeless flies shown in tables 11–15 in the appendix). The appearance of such flies is due to crossing over between the fragments of the third chromosome involved in the translocations and the unbroken third chromosome carrying third-chromosome recessive genes. The following stocks of flies carrying translocations and homozygous with respect to certain third-chromosome recessive genes were established:

a-translocation: $\frac{r_u\ h\ D}{r_u\ h} \;\; \frac{s_r\ e^s\ c_a}{t_h\ s_t\ c_u\ s_r\ e^s\ c_a}$

c-translocation: $\frac{r_u\ h\ t_h\ s_t}{r_u\ h\ t_h\ s_t} \;\; \frac{s_r\ e^s\ c_a}{c_u\ s_r\ e^s\ c_a}$

e-translocation: $\frac{r_u\ h\ t_h\ s_t}{r_u\ h\ t_h\ s_t} \;\; \frac{s_r\ e^s\ c_a}{c_u\ s_r\ e^s\ c_a}$

Using these stocks and the regular stocks of the translocations carrying no third-chromosome recessive genes the following crosses were made:

a-translocation: $\frac{r_u\ h\ D}{r_u\ h} \;\; \frac{s_r\ e^s\ c_a}{t_h\ s_t\ c_u\ s_r\ e^s\ c_a}\ ♀ \times \frac{D}{\quad}\ ♂$

c-translocation: $\frac{r_u\ h\ t_h\ s_t}{r_u\ h\ t_h\ s_t} \;\; \frac{s_r\ e^s\ c_a}{c_u\ s_r\ e^s\ c_a}\ ♀ \times \frac{D}{\quad}\ ♂$

e-translocation: $\frac{r_u\ h\ t_h\ s_t}{r_u\ h\ t_h\ s_t} \;\; \frac{s_r\ e^s\ c_a}{c_u\ s_r\ e^s\ c_a}\ ♀ \times \frac{D}{\quad}\ ♂$

If each of the parents in these crosses produces the six kinds of gametes shown in figure 3, thirty-six classes of zygotes must be produced (see table 7). In c- and e-translocations twenty-eight of these thirty-six classes must die since they carry either a deficiency or a duplication for a section of the third chromosome. The surviving classes are (table 7): Dichaete,

wild type, $D\ s_r\ e^s\ c_a$ and $r_u\ h\ t_h\ s_t$. If the assortment of the fragments of the third chromosome in translocations takes place at random, all the six classes of gametes must be produced in equal numbers. Hence, the frequency of the surviving classes of zygotes must be: $3D$: 3 wild type: 1 $D\ s_r\ e^s\ c_a$: 1 $r_u\ h\ t_h\ s_t$. If no non-disjunctional gametes are produced no $D\ s_r\ e^s\ c_a$ and $r_u\ h\ t_h\ s_t$ individuals will appear. If, finally, the non-disjunctional gametes are produced less frequently than the regular gametes, the frequency of $D\ s_r\ e^s\ c_a$ and $r_u\ h\ t_h\ s_t$ individuals will be lower than indicated above.

TABLE 7

| | (1) | (2) | (3) | (4) | (5) | (6) |
|---|---|---|---|---|---|---|
| | $r_u$ $h$ $t_h$ $s_t$ / $s_r$ $e^s$ $c_a$ | $r_u$ $h$ $t_h$ $s_t$ $c_a$ / $s_r$ $e^s$ $c_a$ | $r_u$ $h$ $t_h$ $s_t$ $c_a$ / $s_r$ $e^s$ $c_a$ | $r_u$ $h$ $t_h$ $s_t$ $c_a$ / $s_r$ $e^s$ $c_a$ | $s_r$ $e^s$ $c_a$ | $r_u$ $h$ $t_h$ $s_t$ $c_a$ / $r_u$ $h$ $t_h$ $s_t$ $c_a$ / $s_r$ $e^s$ $c_a$ |
| (1) $D$ | *Dichaete* | *Dichaete* | dies | dies | dies | dies |
| (2) | *Wild type* | *Wild type* | dies | dies | dies | dies |
| (3) $D$ | dies | dies | dies | $D\ s_r\ e^s\ c_a$ | dies | dies |
| (4) | dies | dies | *Wild type* | dies | dies | dies |
| (5) | dies | dies | dies | dies | dies | $r_u\ h\ t_h\ s_t$ |
| (6) $D$ | dies | dies | dies | dies | *Dichaete* | dies |

The results actually obtained in experiments prove the last assumption, that is, they show that the non-disjunctional gametes are produced, but their frequency is much lower than the frequency of the regular gametes. Indeed, in e-translocation only 29 $r_u\ h\ t_h\ s_t$ and 2 $D\ s_r\ e^s\ c_a$ individuals are observed among 672 flies (table 8). The appearance of the $r_u\ h\ t_h\ s_t$ individuals is due to non-disjunction of the left limb of the third chromosome, whereas the appearance of the $D\ s_r\ e^s\ c_a$ individuals depends upon non-

disjunction of the right limb of the third chromosome (see figure 3 and table 7). In e-translocation the third chromosome is broken to the left of the spindle fibre, that is, the breakage point lies in the left limb of the third chromosome. The higher frequency of $r_u\ h\ t_h\ s_t$ individuals as compared with the $D\ s_r\ e^s\ c_a$ indicates that non-disjunction of the shorter fragment of the third chromosome (which is attached to the fourth chromosome) takes place more frequently than non-disjunction of the longer fragment of the third chromosome (which has retained its own spindle fibre).

In c-translocation the third chromosome is broken to the right of the spindle fibre. By analogy with e-translocation, non-disjunction of the right limb of the third chromosome must take place more frequently than the non-disjunction of the left limb in c-translocation. In other words, $D\ s_r\ e^s\ c_a$ individuals must be more frequent than $r_u\ h\ t_h\ s_t$ individuals. Indeed, 18 $D\ s_r\ e^s\ c_a$ and only 5 $r_u\ h\ t_h\ s_t$ individuals were observed in c-translocation (table 8).

TABLE 8

*Non-disjunction of sections of the third chromosome in translocations.*

Translocation a.

$$\frac{r_u\ h\ D}{r_u\ h} \quad \frac{\quad s_r\ e^s\ c_a}{t_h\ s_t\ c_u\ s_r\ e^s\ c_a}\ ♀ \times \frac{D}{\quad} \quad \frac{\quad}{\quad}\ ♂$$

| Class | Dichaete | Wild type | $r_u\ h\ D$ | $D\ s_r\ e^s\ c_a$ | Total |
|---|---|---|---|---|---|
| Number | 196 | 79 | 31 | 2 | 308 |

Translocation e.

$$\frac{r_u\ h\ t_h\ s_t}{r_u\ h\ t_h\ s_t} \quad \frac{\quad s_r\ e^s\ c_a}{c_u\ s_r\ e^s\ c_a}\ ♀ \times \frac{D}{\quad} \quad \frac{\quad}{\quad}\ ♂$$

| Class | Dichaete | Wild type | $r_u\ h\ t_h\ s_t$ | $D\ s_r\ e^s\ c_a$ | Total |
|---|---|---|---|---|---|
| Number | 305 | 336 | 29 | 2 | 672 |

Translocation c.

$$\frac{r_u\ h\ t_h\ s_t}{r_u\ h\ t_h\ s_t} \quad \frac{\quad s_r\ e^s\ c_a}{c_u\ s_r\ e^s\ c_a}\ ♀ \times \frac{D}{\quad} \quad \frac{\quad}{\quad}\ ♂$$

| Class | Dichaete | Wild type | $r_u\ h\ t_h\ s_t$ | $D\ s_r\ e^s\ c_a$ | Total |
|---|---|---|---|---|---|
| Number | 309 | 374 | 5 | 18 | 706 |

If two individuals both carrying a-translocation and carrying the third chromosomal genes indicated in table 8 are crossed to each other, twenty nine out of the thirty-six possible classes of offspring die. The remaining classes are: 4 $D$ : 1 wild type: 1 $r_u\ h\ D$ : 1 $D\ s_r\ e^s\ c_a$ (the indicated ratio holds true, of course, only in case all six possible classes of the gametes are produced in equal numbers). Actually the ratio 196 $D$ : 79 wild type : 31 $r_u\ h\ D$ : 1 $D\ s_r\ e^s\ c_a$ is observed (table 8). It follows that the non-

disjunctional classes of gametes are produced in a-translocation less frequently than regular gametes. In a-translocations the breakage of the third chomosome occurred at a point located slightly to the right of *D*, that is, in the left limb of the chromosome. Consequently the higher frequency of $r_u\ h$ *D* individuals as compared with $D\ s_r\ e^s\ c_a$ individuals indicates that non-disjunction of the fragment of the third chromosome attached to the fourth takes place more frequently than the non-disjunction of the other fragment of the third chromosome which has retained its own spindle fibre.

Taken as a whole, the results of the experiments just described are important in two respects. First, these results may be explained only on the assumption that the third chromosome in the translocations is broken into two fragments. In other words, the most important result of the cytological investigation of the translocations is proved by independent genetical evidence. Second, the experiments with non-disjunction of the sections of the third chromosome definitely prove that the different fragments of the third chromosome observed in the chromosomal plates of the translocations contain different blocks of genes corresponding to definite sections of the map of the third chromosome.

### NON-DISJUNCTION OF THE FOURTH CHROMOSOMES IN THE TRANSLOCATIONS

Only one free fourth chromosome is seen in most of the chromosomal plates in the translocations. Since the flies carrying translocations are identical with normal flies in appearance and do not possess the characteristics of Haplo-IV, the presence of one more fourth chromosome in their cells is postulated. This fourth chromosome is evidently attached to one of the fragments of the third chromosome. The resulting compound appears in most of the translocations as an unpaired, rod-shaped chromosome in which no dividing line between the fourth chromosome and the fragment of the third is noticeable.

The conjugation of the fourth chromosomes in the translocations must take place in rather unusual conditions, since only one of these chromosomes is free, and the other is permanently attached to the fragment of the third chromosome. Nevertheless, as shown by the genetical behavior of the translocations, the reduction division proceeds more or less normally, and most of the gametes produced carry only one fourth chromosome. That is to say, the free fourth chromosome goes together with the unbroken third chromosome to one pole, and the attached fourth chromosome goes together with both fragments of the broken third chromosome to another pole of the spindle (figure 1). However, the cytological investigation revealed the occurrence of individuals having two free fourth chromosomes

in their cells. Such individuals are especially common in d-translocation, but they are also found in e-translocation. The appearance of such individuals may be explained on the assumption that the non-disjunction of the fourth chromosomes occurs rather frequently in the translocations. The free fourth chromosome might go sometimes together with the attached fourth chromosome and with both fragments of the third chromosome to the same pole of the spindle at the reduction division. Gametes carrying two fourth chromosomes may be produced in this way. Such gametes will give rise to zygotes having three fourth chromosomes, two of the latter being free and one attached to the third chromosome.

Individuals having three fourth chromosomes (triplo-IV) are so little different from normal individuals (diplo-IV) in appearance that their identification on the basis of external characters is impracticable. Nevertheless, the presence of a certain percentage of triplo-IV individuals in the stocks of the translocations may be proved by genetical methods. Two genetical methods were devised to distinguish the triplo-IV individuals from the diplo-IV ones in the translocations.

The first of these methods consists in crossing flies carrying translocations to those carrying the fourth-chromosome gene Minute-IV. Minute-IV is known to be dominant over one dose of the normal allelomorph but recessive to two doses of the normal allelomorph (SCHULTZ 1929). Hence, in the progeny of such a cross, the flies carrying translocations can be at the same time Minute-IV only if they have only two fourth chromosomes (one free and another attached to the third). If the flies carrying translocations have three fourth chromosomes (two free and one attached to the third chromosome), no characteristics of Minute-IV will be manifest.

The second method is based on the fact that in the flies from stocks of the translocations the fourth chromosome attached to the third carries the normal allelomorph of $e_y$, whereas the free fourth chromosome (or the free fourth chromosomes) carry $e_y$. It is clear that if such a fly is crossed to a wild-type fly, and its offspring are then crossed to a fly homozygous for $e_y$, no $e_y$ individuals will appear in the next generation unless the initial fly was a triplo-IV individual.

Both methods just described were used for testing the flies from the stocks of the translocations. The number of the diplo-IV and the triplo-IV flies found is shown in the right-hand column in table 9. The number of the diplo-IV and triplo-IV flies found cytologically is presented in the middle column of table 9.

As seen from table 9, the triplo-IV flies are most frequent in the stock of d-translocation and comparatively rare in the other translocations. The agreement of the results received by the genetical and the cytological methods is satisfactory.

### BEHAVIOR OF THE TRANSLOCATIONS IN HOMOZYGOUS FORM

In the preceding sections we have been dealing with the behavior of the heterozygous translocations. As shown by the cytological data, one of the third chromosomes in the heterozygous translocations is broken into two

TABLE 9

*Number of diplo-IV and triplo-IV flies found in the stocks of the translocations.*

| | CYTOLOGICALLY | | GENETICALLY | |
|---|---|---|---|---|
| | DIPLO-IV | TRIPLO-IV | DIPLO-IV | TRIPLO-IV |
| a-translocation | 15 | .. | 12 | 1 |
| b-translocation | 15 | .. | 12 | 1 |
| c-translocation | 11 | .. | 10 | 2 |
| d-translocation | 4 | 8 | 6 | 14 |
| e-translocation | 11 | 2 | 16 | 2 |

fragments, but the other third chromosome is completely normal. In homozygous translocations two pairs of the fragments of the third chromosome and no normal, unbroken third chromosome must be present. Since some interesting genetical phenomena may arise as a result of such a condition, experiments were planned to secure some of the translocations in homozygous form.

In d-translocation the section of the third chromosome lying to the right of the gene $c_a$ is known to be broken off and attached to the fourth chromosome. Males carrying in the third chromosome involved in the translocation the genes $r_u$, $h$, $t_h$, $s_t$, $c_u$, $s_r$ *and* $e^s$ and carrying in the normal third chromosome the genes $r_u$, $h$, $t_h$, $s_t$, $c_u$, $s_r$, $e^s$ and $c_a$ (that is of the constitution $\frac{r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s}{r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a}$) were crossed to regular (*i.e.*, carrying no translocation) females having in one of their third chromosomes the dominant gene Deformed ($D_f$), the recessive $c_a$ and the crossing over suppressors $C_{III\ R}$ and $C_{III\ L}$. In the $F_1$ generation of this cross Deformed non-claret individuals were selected and inbred. These individuals are heterozygous for the translocation and have the constitution: $\frac{r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s}{C_{III\ L}\ D_f\ C_{III\ R}\ c_a}$. In the next generation ($F_2$) $D_f$ and $r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s$ individuals may be expected to appear

in the ratio 2:1 (the gene $D_f$ is lethal when homozygous). The $r_u\ h\ t_h\ s^t$ $c_u\ s_r\ e^s$ individuals must be homozygous for the translocation. However only $D_f$ individuals were received in $F_2$ (about 1000 flies were examined). In other words, d-translocation is lethal when homozygous.

In e-translocation the third chromosome is broken between the loci of the genes $s_t$ and $p$, and the section containing the genes from $r_u$ to $s_t$ is attached to the fourth chromosome (see figure 2). Males of the constitution $\frac{r_u\ h\ t_h\ s_t \quad s_r\ e^s\ c_a}{r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a}$ were crossed to regular females carrying in one of their third chromosomes the dominant genes $D$ and $S_b$ and the recessives $s_t$, $c_u$ and $e^s$. In the $F_1$ generation the individuals manifesting the characters of $D$, $s_t$, $S_b$ and $e^s$ were selected and inbred. These individuals have the constitution: $\frac{r_u\ h\ t_h\ s_t \quad s_r\ e^s\ c_a}{D \quad s_t\ c_u\ S_b \quad e^s}$. In the $F_2$ generation some $r_u\ h\ t_h\ s_t\ s_r\ e^s\ c_a$ individuals appeared; they must be homozygous for e-translocation.

Individuals homozygous for e-translocation are normal in appearance and have a good viability. However, the females have rudimentary ovaries resembling those of the *Drosophila melanogaster—D. simulans* hybrids (STURTEVANT 1920). Therefore, they are completely sterile when crossed either to males homozygous for e-translocation or to wild-type males. Males homozygous for e-translocation are fertile, though they produce markedly fewer offspring than the wild-type males or males heterozygous for e-translocation.

In c-translocation the third chromosome is broken between the genes $p$ and $c_u$, and the section of the third chromosome containing the genes from $c_u$ to $c_a$ (see figure 2) is attached to the fourth chromosome. Males of the constitution $\frac{r_u\ h\ t_h\ s_t \quad e^s\ c_a}{r_u\ h\ t_h\ s_t \quad c_u\ s_r\ e^s\ c_a}$ were crossed to regular females carrying in one of their third chromosomes the genes $D$, $s_t$, $c_u$, $S_b$ and $e^s$. In the $F_1$ generation $D\ s_t\ S_b\ e^s$ individuals were selected and inbred. They have the constitution $\frac{r_u\ h\ t_h\ s_t \quad e^s\ c_a}{D \quad s_t \quad c_u\ S_b\ e^s}$. In the $F_2$ some $r_u\ h\ t_h\ s_t\ e^s\ c_a$ individuals appeared; they are homozygous for c-translocation. Flies homozygous for c-translocation appear normal somatically, and are fertile in both sexes, though females produce markedly fewer offspring than do wild-type females or females heterozygous for c-translocation.

The behavior of the different translocations in homozygous condition is, therefore, diverse: d-translocation is lethal when homozygous, e-translocation survives but is sterile in one sex, and c-translocation survives and is

fertile in both sexes. (The behavior of a- and b-translocations in homozygous form was not studied because in both of them the third chromosome involved in the translocation carries the dominant gene *D* which is lethal when homozygous. The locus of *D* is so close to the point at which the breakage of the third chromosome occurred in these translocations that it is very difficult to obtain them without the gene *D*.)

The causes which may hinder the viability of the homozygous translocations are obscure. Theoretically, flies heterozygous as well as homozygous for the translocations must have the same genic balance that the normal flies have, and, therefore, must be viable, normal in appearance and in fertility. Indeed, most of the known translocations are normal in heterozygous condition, but, so far as known, only the c-translocation described here approaches the expectation when homozygous.

"Pale" translocation described by BRIDGES is lethal when homozygous (BRIDGES 1923, MORGAN, BRIDGES, STURTEVANT 1925). This fact has been explained by BRIDGES as due to a loss of a fragment of the translocated section during the process of transfer. In other words, the flies homozygous for "Pale" translocation are, according to BRIDGES, homozygous also for a deficiency of a small section of one of the chromosomes involved in the translocation. This explanation is supported by the fact that if the individuals heterozygous for "Pale" translocation are homozygous for the sex-linked gene eosin (an eosin-like eye-color), the resulting color of the eyes is somewhat diluted. Thus, "Pale" translocation has an abnormal genic balance even in heterozygous condition.

Three explanations of the lethal effect (and of the disturbance of the genic balance in general) produced by some of the translocations in homozygous condition may be suggested. First, a lethal or semilethal mutation might arise in one of the chromosomes involved in the translocation. Second, the breakage of the chromosome necessary to produce a translocation might cause some injury to the chromosome involved. For instance, some genes neighboring to the point at which the breakage occurred might be lost or destroyed (see BRIDGES' explanation). Third, it is possible to suppose that the breakage of a chromosome may occur only at a point which has been previously injured by some agent, for instance by the X-ray treatment. The data at present available give no evidence as to which of these explanations is true in the cases of d- and e-translocations.

Females homozygous for c-translocation were studied cytologically. All the chromosomal plates found (plate 2, figures 34–38) contain four pairs of chromosomes. Two pairs out of these four are rod-shaped; one of them is

markedly shorter than the other. Moreover, one pair is V-shaped, and one pair is J-shaped. The J-shaped chromosomes have a subterminal spindle-fibre attachment. Taken as a whole, the chromosomal complex in the homozygous c-translocation is more like that of *Drosophila immigrans* Sturt. (see METZ and MOSES 1923, figure 2 D) or that of *Drosophila melanica* Sturt. (see METZ and MOSES 1923, figure 2 E) than like that of normal *Drosophila melanogaster*.

Nevertheless, the interpretation of the cytological features found in the homozygous translocation is easy if the conditions found in heterozygous c-translocation are taken into account (see plate 1, figures 14–19). The longer pair of the rod-shaped chromosomes found in homozygous translocation are obviously the X-chromosomes. The pair of V-shaped chromosomes are the second chromosomes. The shorter pair of rod-shaped chromosomes and the pair of J-shaped chromosomes are the fragments of the third chromosome. No free fourth chromosome is found in any of the plates studied in homozygous translocation. Obviously, the shorter pair of rod shaped chromosomes consists of the fragments of the third chromosome attached to the fourth chromosomes. Indeed, since only one free fourth chromosome is present in the plates studied in heterozygous c-translocation, no free fourth chromosome must be expected in the chromosomal complex of the homozygous c-translocation.

This interpretation may be tested genetically. If the third chromosome is broken into two separate chromosomes (the rod-shaped and the J-shaped), the genes localized in one of them must show no linkage with the genes localized in the other. In other words, the third-chromosome linkage group of *Drosophila melanogaster* must be broken into two separate linkage groups in the homozygous translocation. The point of the breakage of the third chromosome is located in c-translocation to the right of the spindle fibre, between the genes peach and curled. Hence, the rod-shaped chromosome corresponds to the section of the genetical map of the third chromosome extending from peach to claret; the J-shaped chromosome corresponds to the section extending from scarlet to roughoid (figure 2). The sections just mentioned must behave in the homozygous translocation as two independent linkage groups.

Females homozygous for c-translocation and having the constitution $\frac{r_u\ h\ t_h\ s_t}{r_u\ h\ t_h\ s_t}\ \frac{e^s\ c_a}{e^s\ c_a}$ were crossed to males heterozygous for c-translocation and having the constitution $\frac{D}{}$. In the $F_1$ generation $D$ males were selected and crossed to females homozygous for $r_u$, $h$, $t_h$, $s_t$, $c_u$, $s_r$, $e^s$, $c_a$ and free from

the translocation. These *D*-males must have the constitution $\frac{D}{r_u\ h\ t_h\ s_t\ \ e^s\ c_a}$, that is, they are homozygous for the translocation but heterozygous for the third-chromosomal genes indicated. Should the third chromosomes in the homozygous translocation not be broken, only *D* and $r_u\ h\ t_h\ s_t\ e^s\ c_t$ individuals would appear in the offspring of this cross. However, if the third-chromosome linkage group, following the breakage of the third chromosome, is also broken into two independent linkage groups, *D*, $r_u\ h\ t_h\ s_t\ e^s\ c_a$, $r_u\ h\ t_h\ s_t$ and $D\ e^s\ c_a$ individuals will appear in the ratio 1:1:1:1. The results obtained in the experiment are:

| $D$ | $r_u\ h\ t_h\ s_t$ | $D\ e^s\ c_a$ | $r_u\ h\ t_h\ s_t\ e^s\ c_a$ | Total |
|---|---|---|---|---|
| 232 | 243 | 238 | 227 | 940 |

Hence, two "new" linkage groups of genes replace, in the homozygous translocation, the original third-chromosome linkage group of *Drosophila melanogaster*. If females homozygous for c-translocation and having the constitution $\frac{D}{r_u\ h\ t_h\ s_t\ \ e^s\ c_a}$ are crossed to homozygous $r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ males free from the translocation, the two "new" linkage groups remain independent from each other, but some crossing over occurs within each of them. The frequency of crossing over in the "new" linkage groups is shown in table 10 which is based on the counts of 752 flies.

TABLE 10

*Frequency of crossing over in the "new" linkage groups in the homozygous translocation.*

| | INTERVAL | FREQUENCY IN HOMOZYGOUS TRANSLOCATION—PERCENT | STANDARD FREQUENCY PERCENT | DIFFERENCE |
|---|---|---|---|---|
| The J-shaped chromosome | $r_u$-$h$ | 27.8 | 26.5 | +1.3 |
| | $h$-$D$ | 14.4 | 13.9 | +0.5 |
| | $D$-$t_h$ | 1.2 | 1.8 | −0.6 |
| | $t_h$-$s_t$ | 1.5 | 1.8 | −0.3 |
| | $s_t$-$e^s$ | 46.5 | 26.7 | +19.8 |
| The rod-shaped chromosome | $e^s$-$c_a$ | 31.1 | 30.0 | +1.1 |

It may be concluded that the frequencies of crossing over in the J-shaped chromosome are approximately equal to the frequencies of crossing over in the corresponding section of the normal, unbroken, third chromosome. The same is true in respect to the frequency of crossing over in the

$e^s$–$c_a$ interval in the rod-shaped chromosome. Whether the frequency of crossing over in the whole rod-shaped chromosome is also equal to that in the corresponding section of the normal third chromosome, can not be decided on the basis of the data at hand. It may conceivably be different, since the rod-shaped chromosome in c-translocation contains not only the material corresponding to a section of the third chromosome, but also that corresponding to the fourth chromosome of the normal fly. The presence of the fourth-chromosome material in the rod-shaped chromosome might alter the frequency of crossing over in the neighboring part of the third chromosome as compared with the normal condition. This question needs further study.

In any case, the data presented here show that the classical assumption, according to which each linkage group corresponds to a separate chromosome, is correct.

## DISCUSSION OF THE RESULTS

The cytological study of the translocations has shown that in each of them the third chromosome is broken into two fragments of unequal length and that the shorter fragment is attached to the fourth chromosome. The non-disjunction of the sections of the third chromosome in the translocations furnishes a genetical proof of the breakage of the third chromosomes. Finally, the results of the observations on the behavior of the homozygous translocation must be evaluated as the most convincing evidence in favor of our interpretation of the translocations.

The loci at which the breakage of the third chromosome took place in each of the translocations are determined by two completely independent methods. The first of these methods is a purely genetical one: the study of the linkage relations of the genes belonging to the third and to the fourth linkage groups. The location of the break is determined by this method in terms of units of map-distance, by essentially the same procedure as that by which the location of a mutant gene in the third chromosome of Drosophila is usually studied. The relative distances between the breaking-points in the different translocations may be compared in the same way in which the relative distances between the genes located in the third chromosome may be compared (figure 2). In doing so we proceed on the same basic assumption on which the genetical "map" of the third chromosome or the genetical map of any other chromosome is built. That is the assumption that the genes within a chromosome are arranged in linear order, and that the frequency of crossing over between any two genes

located in the same chromosome is a function of the distance between these genes.

The second method of determination of the locus at which the breakage of the chromosome took place is the direct investigation of the length of the fragments of the chromosome in cytological preparations. Since the shape of the chromosome during the metaphase stage is relatively constant, the location of the breakages may be expressed in terms of their distance from the ends of the chromosome, from the spindle fibre, from the constrictions, and from each other.

Assuming that the theory of linear arrangement of the genes within the chromosome is correct, the cytological conditions may be predicted on the basis of the knowledge of the genetical location of the breaking-point and vice versa. It has been shown in the preceding sections that such a prediction is really possible. In a- and b-translocations the loci of breakage as determined genetically nearly coincide with each other (figure 2). The chromosomes of a-translocation are nearly indistinguishable from those of b-translocation. In e-translocation the locus of the breakage on the basis of the genetical data must be supposed to lie between that observed in a- and b-translocations and the spindle fibre. This is exactly what has been found cytologically in e-translocation. The genetical data show that only a very small piece of the third chromosome may be attached to the fourth in d-translocation. Correspondingly, only a slight increase of one of the fourth chromosomes is observed cytologically. On the basis of the genetical evidence the third chromosome is broken in c-translocation at approximately the same distance from the spindle fibre as in e-translocation. The fragments of the third chromosome observed cytologically in c-translocation are but slightly different in size from those observed in e-translocation.

There is no escape from the conclusion that the sequence of the third-chromosomal genes as represented by the genetical map of this chromosome is the same as their sequence in the chromosome studied cytologically. In other words the genetical map represents the actual sequence of genes within the chromosome. Thus, the theory of the linear arrangement of the genes receives a strong basis of cytological evidence.

If the question about the reality of the linear order of genes within the chromosome is answered in the affirmative, it becomes possible to raise another question. Are the relative distances between the genes correctly represented by the genetical map of the third chromosome, or does the

genetical map give a somewhat distorted image of the actual spatial relations of the genes?

The cytologically observed points at which the third chromosome is broken in different translocations form a skeleton of a "cytological map" of the third chromosome. Furthermore, since certain genes are known to be adjacent to the points at which the breakage of the chromosome has occurred in different translocations, it seems to be justified to conclude that these genes are located in the chromosome in the neighborhood of the observed breakage-points. Thus, the "cytological map" represents the loci of the observed breakages, and, by inference, the loci of some of the third-chromosomal genes. The genetical and the cytological maps of the third chromosome, drawn to the same scale, are shown in figure 4.

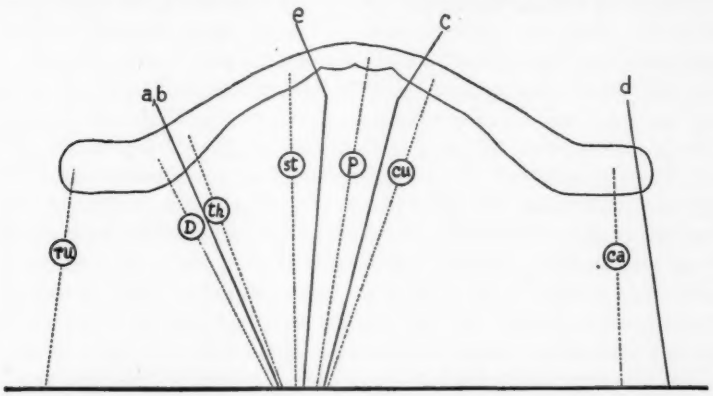


FIGURE 4.—Comparison of a genetical (below) and a cytological (above) map of the third chromosome of *Drosophila melanogaster*. a, b, c, d and e—the observed breaking-points. $r_u$—roughoid; *D*—Dichaete; $t_h$—thread; $s_t$—scarlet; *p*—peach; $c_u$—curled; $c_a$—claret.

It must be emphasized, that the cytological map presented in figure 4 is far less exact than the standard genetical map. First of all, the exact measurement of the fragments of the chromosomes of Drosophila is exceedingly difficult because of their extreme smallness. Furthermore, the presence of the fourth chromosome attached to one of the fragments of the third chromosome makes the estimation of the size of the latter still less exact. A certain alteration of the shape of the fragments of the third chromosome after breakage is also possible. Finally, the number of breakages of the third chromosome so far observed is too small to allow the construction of a detailed cytological map. Nevertheless, the relative distances

between the genes as represented by the genetical and the cytological map are so widely different, that certain conclusions may be drawn even on the basis of study of the cytological map available at present.

The distance between the locus of the breakage observed in a-translocation and the left-hand end of the third chromosome (gene roughoid) equals (at least) 41 map-units. The distance between the breakage observed in a-translocation and that observed in e-translocation equals only about 4 map-units. In other words, the distance between the left end of the third chromosome and the breakage observed in a-translocation is at least ten times longer than the distance between the breakages observed in a- and in e-translocations. That is the determination based on the genetical data. However, the cytological map shows that the latter distance is actually longer than the former. If studied genetically, the distance between the breakages observed in e- and c-translocations equals about 5 map-units, as compared with the 40 (or more) units distance between the breaking-point observed in a-translocation and the left-hand end of the third chromosome. Cytologically these distances are about equal in length. The distance between the point of the breakage observed in c-translocation and the right-hand end of the third chromosome is at least 60 units long; the distance between the points of breakage observed in e- and in c-translocations is about 5 units long. Cytologically, the former distance is only about twice as long as the latter (see figure 4).

PAINTER and MULLER (1929) have shown that the section of the third chromosome extending from sooty to the right-hand end of the third chromosome (this section must be at least 35 units long) is actually so small that it becomes scarcely visible if attached to the second chromosome.

Thus, the discrepancies between the length of the different sections of the third chromosome estimated on the basis of the genetical data and their length actually observed are very great. All these discrepancies may, however, be generalized in a form which discloses a very interesting regularity. The distances between the loci of the breakages or of the genes lying in the middle part of the chromosome are longer cytologically than they might be expected to be on the basis of the genetical map. On the other hand, the distances between the loci lying near the ends of the chromosome are shorter cytologically than they might be expected to be on the basis of the genetical data. The genetical map represents the genes located in the middle part of the chromosome (near the spindle fibre) relatively too close to each other, and the genes located at the ends of the chromosomes relatively too far apart.

In the metaphase stage the ends of the third chromosome appear to be considerably thicker than the middle part of the chromosome. This is partly due to a stronger condensation of the material located near the ends of the chromosome as compared with the material located near its middle, and partly to a premature splitting of the ends of the chromosome (compare figure 5, plate 1, representing the chromosome in the late prophase stage with the other figures representing chromosomes in the metaphase stage). This may suggest that the distances between the genes located near the ends of the chromosome are relatively shorter when the chromosome is in the metaphase stage than they are in the stage when crossing over takes place. However, the discrepancies between the relative distances of the genes from each other shown by the genetical and the cytological maps are too great to be accounted for by the different diameter of the different parts of the third chromosome.

The distances between the genes on the genetical map represent nothing but the frequency of crossing over between these genes. There is, however, no evidence that the frequency of crossing over between any two genes must be necessarily proportional to the absolute distance between these genes in the chromosome. In fact, there exists some evidence against such an assumption. The genes are not distributed at random along the genetical maps of the chromosomes but are crowded in some regions and sparse in others. This fact leads to the hypothesis according to which the frequency of crossing over per unit of the absolute distance may be different in different regions of the same chromosome (MORGAN, BRIDGES, STURTEVANT 1925, p. 93). The map of the third chromosome shows that the genes are crowded in the region neighboring to the spindle-fibre attachment but both ends of the map have few genes and long intervals in which no genes are known (figure 2).

If we suppose that the frequency of crossing over per unit of the absolute distance is much higher near the ends of the third chromosome than near the spindle fibre, the observed discrepancies between the genetical and the cytological maps of this chromosome find a simple explanation. The genetical map of the third chromosome represents correctly the actual sequence of the genes within the chromosome. However, the distances between the genes shown by the genetical map are functions of the actual distance between them in the chromosome as well as of the regional differences in the frequency of crossing over. In its turn, the frequency of crossing over per unit of the absolute distance in a given region seems to be a function of the distance between this region and the locus of the spindle-fibre attachment.

A similar conclusion is reached by PAINTER and MULLER (1929) on the basis of study of the deletions in the X-chromosome of *Drosophila melanogaster*. In this case the cytological data indicate that the actual distances between the genes located in the left-hand end of the X-chromosome are relatively longer than the distances between these genes suggested by the genetical map of this chromosome. The genetical map of the X-chromosome shows that the genes are strongly crowded at the left-hand end of the chromosome.

It is fairly certain on the basis of the data at hand that the genes are distributed at random along the genetical map of the third chromosome of *Drosophila melanogaster*, or at least much more nearly so than on the genetical map. Both the genetical and the cytological maps show only those genes which have given mutations. It follows that the process of mutation affects all the parts of the chromosome more or less at random.

## SUMMARY

1. Five cases of translocation involving the third and the fourth chromosomes were observed in the progeny of flies treated by X-rays. In each of the five cases a section of the third chromosome became broken off and attached to the fourth chromosome.

2. Flies carrying translocations in heterozygous form are apparently normal in appearance and in viability, and nearly normal in fertility.

3. The loci at which the breakage of the third chromosome took place in each of the translocations were determined by studying the linkage between the genes of the third and those of the fourth chromosome linkage groups, which in the translocations behave as if they were localized in the same chromosome. The loci of breakage of the third chromosome are different in different translocations.

4. The frequency of crossing over is markedly decreased in the limb of the third chromosome in which the breakage has occurred, and slightly increased in the opposite limb. The point dividing the two limbs (the point of the spindle-fibre attachment) is slightly to the left of the locus of peach.

5. Non-disjunction of the sections of the third chromosome occurs in the translocations and can be detected by a suitable genetical method (see text). The study of the non-disjunction of the sections of the third chromosome gives a proof of the presence of the breakages of the third chromosome in the translocations.

6. Non-disjunction of the fourth chromosomes also occurs in the translocations.

7. The cytological investigation has shown that the longer pair of the V-shaped chromosomes is that affected by the translocations. It may be concluded that the longer pair of the V-shaped autosomes of *Drosophila melanogaster* carries the third-chromosome linkage group, and the shorter pair of the V-shaped autosomes carries the second-chromosome linkage group of the genes.

8. One of the third chromosomes is found cytologically to be broken into fragments in the translocations. One of the fragments is apparently attached to the fourth chromosome, since only one free fourth chromosome is present in most plates studied.

9. The length of the fragments of the third chromosome observed in cytological preparations is roughly proportional to their length suggested by the genetical data. This fact may be considered as a cytological proof of the theory of linear arrangement of the genes within the chromosomes.

10. A "cytological map" of the third chromosome is constructed on the basis of the cytological study of the translocations (see figure 4 in text). The comparison of the cytological map with the regular genetical map of the third chromosome shows that the distances between the genes located in the middle part of the chromosome are larger cytologically than they might be expected to be on the basis of the genetical map. This is apparently due to the low frequency of crossing over per unit of absolute distance in the middle part of the third chromosome, and to the high frequency of crossing over near the ends of the chromosome.

11. Two of the translocations were obtained in homozygous form. Individuals homozygous for the translocations are normal in appearance but sometimes sterile in the female.

12. Cytological study of the homozygous translocation showed, according to expectation, that both third chromosomes are broken into two fragments. No free fourth chromosome is found in homozygous translocation. Hence, the homozygous translocation possesses four pairs of chromosomes but two of them are very different in appearance from the chromosomes found in the normal fly.

13. The third-chromosome linkage group of *Drosophila melanogaster* is broken in homozygous translocation into two independent linkage groups of genes corresponding to the different sections of the normal third chromosome. This is, undoubtedly, due to the breakage of the third chromosome.

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## APPENDIX—TABLES 11–17.

### TABLE 11

*Translocation a.*

$$\frac{e_y}{\cdots}\ \frac{r_u\ h\,2\ 3\ 4\ l_h\ s_t\ c_u\ s_r\ e^s\ c_a}{1\ D+\ 5\ 6\ 7\ 8\ 9} \times e_y\, r_u\, h\, l_h\, s_t\, c_u\, s_r\, e^s\, c_a\ \delta^{\text{n}}.$$

| CLASSES OF THE OFFSPRING | NUMBER OBSERVED | CLASSES OF THE OFFSPRING | NUMBER OBSERVED |
|---|---|---|---|
| 0 $D$ | 508 | 8,9 $D\,e^s$ | 17 |
| 0 $e_y\, r_u\, h\, l_h\, s_t\, c_u\, s_r\, e^s\, c_a$ | 143 | 8,9 $e_y\, r_u\, h\, l_h\, s_t\, c_u\, s_r\, c_a$ | 3 |
| Total non-crossovers | 651 | 1,8 $r_u\, D\, e^s\, c_a$ | 26 |
| | | 1,8 $e_y\, h\, l_h\, s_t\, c_u\, s_r$ | 4 |
| 1 $D\, r_u$ | 140 | 2,8 $r_u\, h\, D\, e^s\, c_a$ | 2 |
| 1 $e_y\, h\, l_h\, s_t\, c_u\, s_r\, e^s\, c_a$ | 33 | 2,8 $e_y\, l_h\, s_t\, c_u\, s_r$ | 1 |
| 2 $D\, r_u\, h$ | 24 | 4,8 $D\, l_h\, s_t\, c_u\, s_r$ | 1 |
| 2 $e_y\, l_h\, s_t\, c_u\, s_r\, e^s\, c_a$ | 11 | 6,8 $D\, c_u\, s_r$ | 9 |
| 3 $e_y\, D\, l_h\, s_t\, c_u\, s_r\, e^s\, c_a$ | 1 | 6,8 $e_y\, r_u\, h\, l_h\, s_t\, e^s\, c_a$ | 4 |
| 4 $e_y\, r_u\, h$ | 1 | 1,7 $r_u\, D\, s_r\, e^s\, c_a$ | 22 |
| 5 $D\, s_t\, c_u\, s_r\, e^s\, c_a$ | 1 | 1,7 $e_y\, h\, l_h\, s_t\, c_u$ | 14 |
| 5 $e_y\, r_u\, h\, l_h$ | 2 | 2,7 $r_u\, h\, D\, s_r\, e^s\, c_a$ | 4 |
| 6 $D\, c_u\, s_r\, e^s\, c_a$ | 55 | 2,7 $e_y\, l_h\, s_t\, c_u$ | 4 |
| 6 $e_y\, r_u\, h\, l_h\, s_t$ | 25 | 6,7 $D\, c_u$ | 2 |
| 7 $D\, s_r\, e^s\, c_a$ | 156 | 6,7 $e_y\, r_u\, h\, l_h\, s_t\, s_r\, e^s\, c_a$ | 3 |
| 7 $e_y\, r_u\, h\, l_h\, s_t\, c_u$ | 56 | 5,7 $e_y\, r_u\, h\, l_h\, s_r\, e^s\, c^s$ | 2 |
| 8 $D\, e^s\, c_a$ | 120 | 1,6 $r_u\, D\, c_u\, s_r\, e^s\, c_a$ | 5 |
| 8 $e_y\, r_u\, h\, l_h\, s_t\, c_u\, s_r$ | 54 | 1,6 $e_y\, h\, l_h\, s_t$ | 1 |
| 9 $D\, c_a$ | 318 | 2,6 $r_u\, h\, D\, c_u\, s_r\, e^s\, c_a$ | 1 |
| 9 $e_y\, r_u\, h\, l_h\, s_t\, c_u\, s_r\, e^s$ | 83 | | |
| Total single-crossovers | 1080 | Total double crossovers | 332 |
| 1,9 $r_u\, D\, c_a$ | 82 | 1,7,9 $r_u\, D\, s_r\, e^s$ | 8 |
| 1,9 $e_y\, h\, l_h\, s_t\, c_u\, s_r\, e^s$ | 13 | 1,7,9 $e_y\, h\, l_h\, s_t\, c_u\, c_a$ | 4 |
| 2,9 $r_u\, h\, D\, c_a$ | 7 | 1,6,9 $r_u\, D\, c_u\, s_r\, e^s$ | 3 |
| 2,9 $e_y\, l_h\, s_t\, c_u\, s_r\, e^s$ | 7 | 1,6,9 $e_y\, h\, l_h\, s_t\, c_a$ | 1 |
| 6,9 $D\, c_u\, s_r\, e^s$ | 12 | 1,6,8 $r_u\, D\, c_u\, s_r$ | 1 |
| 6,9 $e_y\, r_u\, h\, l_h\, s_t\, c_a$ | 15 | 1,8,9 $r_u\, D\, e^s$ | 1 |
| 7,9 $D\, s_r\, e^s$ | 52 | Total triple crossovers | 18 |
| 7,9 $e_y\, r_u\, h\, l_h\, s_t\, c_u\, c_a$ | 19 | Grand total | 2081 |

TABLE 12

*Translocation b.*

$e_y\ r_u\ h\ 2\ 3\ 4\ l_h\ s_l\ c_u\ s_r\ e^s\ c_a$ ♀ × $e_y\ r_u\ h\ l_h\ s_l\ c_u\ s_r\ e^s\ c_a$ ♂.

$\ldots\ 1\ \ D+\ \ 5\ 6\ 7\ 8\ 9$

| CLASSES OF THE OFFSPRING | NUMBER OBSERVED | CLASSES OF THE OFFSPRING | NUMBER OBSERVED |
|---|---|---|---|
| 0 $D$ | 304 | 6, 9 $D\ c_u\ s_r\ e^s$ | 7 |
| 0 $e_y\ r_u\ h\ l_h\ s_l\ c_u\ s_r\ e^s\ c_a$ | 110 | 6, 9 $e_y\ r_u\ h\ l_h\ s_l\ c_a$ | 9 |
| Total non-crossovers | 414 | | |
| 1 $r_u\ D$ | 129 | 7, 9 $D\ s_r\ e^s$ | 22 |
| 1 $e_y\ h\ l_h\ s_l\ c_u\ s_r\ e^s\ c_a$ | 22 | 7, 9 $e_y\ r_u\ h\ l_h\ s_l\ c_u\ c_a$ | 12 |
| 2 $r_u\ h\ D$ | 27 | 8, 9 $D\ e^s$ | 9 |
| 2 $e_y\ l_h\ s_l\ c_u\ s_r\ e^s\ c_a$ | 18 | 8, 9 $e_y\ r_u\ h\ l_h\ s_l\ c_u\ s_r\ c_a$ | 1 |
| 3 $r_u\ h$ | 1 | 1, 8 $r_u\ D\ e^s\ c_a$ | 7 |
| 3 $e_y\ D\ l_h\ s_l\ c_u\ s_r\ e^s\ c_a$ | 1 | 1, 8 $e_y\ h\ l_h\ s_l\ c_u\ s_r$ | 4 |
| 5 $D\ s_l\ c_u\ s_r\ e^s\ c_a$ | 1 | 2, 8 $r_u\ h\ D\ e^s\ c_a$ | 5 |
| 5 $e_y\ r_u\ h\ l_h$ | 4 | 2, 8 $e_y\ l_h\ s_l\ c_u\ s_r$ | 1 |
| 6 $D\ c_u\ s_r\ e^s\ c_a$ | 20 | 6, 8 $D\ c_u\ s_r$ | 5 |
| 6 $e_y\ r_u\ h\ l_h\ s_l$ | 17 | 6, 8 $e_y\ r_u\ h\ l_h\ s_l\ e^s\ c_a$ | 3 |
| 7 $D\ s_r\ e^s\ c_a$ | 114 | 1, 7 $D\ r_u\ s_r\ e^s\ c_a$ | 36 |
| 7 $e_y\ r_u\ h\ l_h\ s_l\ c_u$ | 35 | 1, 7 $e_y\ h\ l_h\ s_l\ c_u$ | 10 |
| 8 $D\ e^s\ c_a$ | 69 | 2, 7 $r_u\ h\ D\ s_r\ e^s\ c_a$ | 8 |
| 8 $e_y\ r_u\ h\ l_h\ s_l\ c_u\ s_r$ | 37 | 2, 7 $e_y\ l_h\ s_l\ c_u$ | 3 |
| 9 $D\ c_a$ | 202 | 1, 6 $r_u\ D\ c_u\ s_r\ e^s\ c_a$ | 5 |
| 9 $e_y\ r_u\ h\ l_h\ s_l\ c_u\ s_r\ e^s$ | 71 | 2, 6 $r_u\ h\ D\ c_u\ s_r\ e^s\ c_a$ | 1 |
| | | 1, 2 $h\ D$ | 1 |
| Total single crossovers | 768 | Total double crossovers | 255 |
| 1, 9 $r_u\ D\ c_a$ | 45 | 1, 7, 9 $r_u\ D\ s_r\ e^s$ | 5 |
| 1, 9 $e_y\ h\ l_h\ s_l\ c_u\ s_r\ e^s$ | 40 | 2, 7, 9 $r_u\ h\ D\ s_r\ e^s$ | 1 |
| 2, 9 $r_u\ h\ D\ c_a$ | 16 | 1, 8, 9 $r_u\ D\ e^s$ | 2 |
| 2, 9 $e_y\ l_h\ s_l\ c_u\ s_r\ e^s$ | 4 | 7, 8, 9 $e_y\ r_u\ h\ l_h\ s_l\ c_u\ e^s$ | 1 |
| 3, 9 $r_u\ h\ c_a$ | 1 | 1, 2, 7 $e_y\ r_u\ l_h\ s_l\ c_u$ | 1 |
| | | Total triple crossovers | 10 |
| | | Grand total | 1447 |

TABLE 13

*Translocation c.*

$e_y\ r_u\ h\ 2\ 3\ t_h\ s_t\ 5\ 6\ c_u\ s_r\ e^s\ c_a$ ♀ × $e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ ♂.

$\cdot\cdot\ 1\ D\ 4\ +\ 7\ 8\ 9$

| CLASSES OF THE OFFSPRING | | NUMBER OBSERVED |
|---|---|---|
| 0 | $D$ | 675 |
| | $e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ | 240 |
| Total non-crossovers | | 915 |
| 1 | $r_u\ D$ | 396 |
| | $e_y\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ | 143 |
| 2 | $r_u\ h\ D$ | 198 |
| | $e_y\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ | 116 |
| 3 | $r_u\ h$ | 18 |
| | $e_y\ D\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ | 5 |
| 4 | $r_u\ h\ t_h$ | 11 |
| | $e_y\ D\ s_t\ c_u\ s_r\ e^s\ c_a$ | 11 |
| 5 | $r_u\ h\ t_h\ s_t$ | 37 |
| | $e_y\ D\ c_u\ s_r\ e^s\ c_a$ | 26 |
| 6 | $e_y\ r_u\ h\ t_h\ s_t$ | 8 |
| | $D\ c_u\ s_r\ e^s\ c_a$ | 12 |
| 7 | $e_y\ r_u\ h\ t_h\ s_t\ c_u$ | 12 |
| | $D\ s_r\ e^s\ c_a$ | 50 |
| 8 | $e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r$ | 51 |
| | $D\ e^s\ c_a$ | 96 |
| 9 | $e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s$ | 133 |
| | $D\ c_a$ | 384 |
| Total single crossovers | | 1707 |
| 1, 9 | $r_u\ D\ c_a$ | 169 |
| | $e_y\ h\ t_h\ s_t\ c_u\ s_r\ e^s$ | 48 |
| 2, 9 | $r_u\ h\ D\ c_a$ | 79 |
| | $e_y\ t_h\ s_t\ c_u\ s_r\ e^s$ | 35 |
| 3, 9 | $r_u\ h\ c_a$ | 15 |
| | $e_y\ D\ t_h\ s_t\ c_u\ s_r\ e^s$ | 2 |
| 4, 9 | $r_u\ h\ t_h\ c_a$ | 2 |
| 5, 9 | $e_y\ D\ c_u\ s_r\ e^s$ | 4 |
| | $r_u\ h\ t_h\ s_t\ c_a$ | 23 |
| 6, 9 | $D\ c_u\ s_r\ e^s$ | 1 |
| 7, 9 | $D\ s_r\ e^s$ | 8 |
| | $e_y\ r_u\ h\ t_h\ s_t\ c_u\ c_a$ | 4 |
| 8, 9 | $D\ e^s$ | 6 |
| | $e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r\ c_a$ | 1 |
| 1, 8 | $r_u\ D\ e^s\ c_a$ | 40 |
| | $e_y\ h\ t_h\ s_t\ c_u\ s_r$ | 6 |
| 2, 8 | $r_u\ h\ D\ e^s\ c_a$ | 16 |
| | $e_y\ t_h\ s_t\ c_u\ s_r$ | 11 |
| 3, 8 | $r_u\ h\ e^s\ c_a$ | 3 |
| 4, 8 | $r_u\ h\ t_h\ e^s\ c_a$ | 1 |
| 5, 8 | $e_y\ D\ c_u\ s_r$ | 2 |
| | $r_u\ h\ t_h\ s_t\ e^s\ c_a$ | 4 |
| 6, 8 | $D\ c_u\ s_r$ | 1 |
| 1, 7 | $r_u\ D\ s_r\ e^s\ c_a$ | 22 |
| | $e_y\ h\ t_h\ s_t\ c_u$ | 1 |
| 2, 7 | $r_u\ h\ D\ s_r\ e^s\ c_a$ | 10 |
| | $e_y\ t_h\ s_t\ c_u$ | 2 |
| 3, 7 | $r_u\ h\ s_r\ e^s\ c_a$ | 2 |
| 4, 7 | $r_u\ h\ t_h\ s_r\ e^s\ c_a$ | 3 |
| 1, 5 | $e_y\ r_u\ D\ c_u\ s_r\ e^s\ c_a$ | 4 |
| | $h\ t_h\ s_t$ | 10 |
| 2, 5 | $e_y\ r_u\ h\ D\ c_u\ s_r\ e^s\ c_a$ | 1 |
| | $t_h\ s_t$ | 5 |
| 3, 5 | $D\ t_h\ s_t$ | 2 |
| 1, 2 | $h\ D$ | 9 |
| | $e_y\ r_u\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ | 1 |
| Total double crossovers | | 553 |
| 1, 2, 9 | $h\ D\ c_a$ | 3 |
| 2, 7, 9 | $r_u\ h\ D\ s_r\ e^s$ | 1 |
| 1, 7, 9 | $r_u\ D\ s_r\ e^s$ | 1 |
| 1, 5, 9 | $h\ t_h\ s_t\ c_a$ | 2 |
| 2, 5, 9 | $t_h\ s_t\ c_a$ | 1 |
| 2, 6, 8 | $r_u\ h\ D\ c_u\ s_r$ | 1 |
| 1, 5, 7 | $h\ t_h\ s_t\ s_r\ e^s\ c_a$ | 1 |
| Total triple crossovers | | 10 |
| Grand total | | 3185 |

TABLE 14

*Translocation d.*

$e_y\ r_u\ h\ 2\ 3\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a\ 9$

$\underline{\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad}$ ♀ × $e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ ♂.

$..\quad 1\quad D\quad 4\ 5\ 6\ 7\ 8\ +$

| CLASSES OF THE OFFSPRING | | NUMBER OBSERVED |
|---|---|---|
| 0 | $D$ | 289 |
| | $e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ | 152 |
| Total non-crossovers | | 441 |
| 1 | $D\ r_u$ | 185 |
| | $e_y\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ | 99 |
| 2 | $D\ r_u\ h$ | 77 |
| | $e_y\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ | 81 |
| 3 | $r_u\ h$ | 6 |
| | $e_y\ D\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ | 5 |
| 4 | $r_u\ h\ t_h$ | 3 |
| | $e_y\ D\ s_t\ c_u\ s_r\ e^s\ c_a$ | 7 |
| 5 | $r_u\ h\ t_h\ s_t$ | 29 |
| | $e_y\ D\ c_u\ s_r\ e^s\ c_a$ | 28 |
| 6 | $r_u\ h\ t_h\ s_t\ c_u$ | 41 |
| | $e_y\ D\ s_r\ e^s\ c_a$ | 72 |
| 7 | $r_u\ h\ t_h\ s_t\ c_u\ s_r$ | 37 |
| | $e_y\ D\ e^s\ c_a$ | 48 |
| 8 | $r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s$ | 82 |
| | $e_y\ D\ c_a$ | 79 |
| 9 | $r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ | 8 |
| | $e_y\ D$ | 21 |
| Total single crossovers | | 908 |
| 1, 9 | $e_y\ r_u\ D$ | 17 |
| | $h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ | 4 |
| 2, 9 | $e_y\ r_u\ h\ D$ | 6 |
| | $t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ | 1 |
| 4, 9 | $D\ s_t\ c_u\ s_r\ e^s\ c_a$ | 1 |
| 5, 9 | $D\ c_u\ s_r\ e^s\ c_a$ | 2 |
| | $e_y\ r_u\ h\ t_h\ s_t$ | 1 |
| 6, 9 | $D\ s_r\ e^s\ c_a$ | 8 |
| 8, 9 | $D\ c_a$ | 5 |
| | $e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s$ | 9 |
| 1, 8 | $h\ t_h\ s_t\ c_u\ s_r\ e^s$ | 31 |
| | $e_y\ r_u\ D\ c_a$ | 51 |
| 2, 8 | $t_h\ s_t\ c_u\ s_r\ e^s$ | 26 |
| | $e_y\ r_u\ h\ D\ c_a$ | 13 |
| 3, 8 | $D\ t_h\ s_t\ c_u\ s_r\ e^s$ | 2 |
| | $e_y\ r_u\ h\ c_a$ | 1 |
| 4, 8 | $D\ s_t\ c_u\ s_r\ e^s$ | 4 |
| 5, 8 | $D\ c_u\ s_r\ e^s$ | 8 |

| CLASSES OF THE OFFSPRING | | NUMBER OBSERVED |
|---|---|---|
| 5, 8 | $e_y\ r_u\ h\ t_h\ s_t\ c_a$ | 3 |
| 6, 8 | $D\ s_r\ e^s$ | 7 |
| 7, 8 | $D\ e^s$ | 1 |
| 1, 7 | $e_y\ r_u\ D\ e^s\ c_a$ | 32 |
| | $h\ t_h\ s_t\ c_u\ s_r$ | 11 |
| 2, 7 | $e_y\ r_u\ h\ D\ e^s\ c_a$ | 10 |
| | $t_h\ s_t\ c_u\ s_r$ | 4 |
| 5, 7 | $D\ c_u\ s_r$ | 2 |
| | $e_y\ r_u\ h\ t_h\ s_t\ e^s\ c_a$ | 3 |
| 1, 6 | $h\ t_h\ s_t\ c_u$ | 14 |
| | $e_y\ r_u\ D\ s_r\ e^s\ c_a$ | 38 |
| 2, 6 | $t_h\ s_t\ c_a$ | 13 |
| | $e_y\ r_u\ h\ D\ s_r\ e^s\ c_a$ | 14 |
| 3, 6 | $D\ t_h\ s_t\ c_u$ | 1 |
| | $e_y\ r_u\ h\ s_r\ e^s\ c_a$ | 3 |
| 4, 6 | $e_y\ r_u\ h\ t_h\ s_r\ e^s\ c_a$ | 1 |
| 5, 6 | $e_y\ r_u\ h\ t_h\ s_t\ s_r\ e^s\ c_a$ | 1 |
| 1, 5 | $h\ t_h\ s_t$ | 6 |
| | $e_y\ r_u\ D\ c_u\ s_r\ e^s\ c_a$ | 6 |
| 2, 5 | $t_h\ s_t$ | 4 |
| | $e_y\ r_u\ h\ D\ c_u\ s_r\ e^s\ c_a$ | 1 |
| 3, 5 | $D\ t_h\ s_t$ | 1 |
| 1, 2 | $h\ D$ | 1 |
| Total double crossovers | | 367 |
| 1, 8, 9 | $e_y\ h\ t_h\ s_t\ c_u\ s_r\ e^s$ | 1 |
| 1, 5, 9 | $e_y\ h\ t_h\ s_t$ | 1 |
| 5, 8, 9 | $e_y\ D\ c_u\ s_r\ e^s$ | 1 |
| 7, 8, 9 | $e_y\ D\ e^s$ | 1 |
| 1, 2, 8 | $r_u\ t_h\ s_t\ c_u\ s_r\ e^s$ | 1 |
| 1, 6, 8 | $r_u\ D\ s_r\ e^s$ | 1 |
| 2, 6, 8 | $r_u\ h\ D\ s_r\ e^s$ | 4 |
| 1, 2, 7 | $r_u\ t_h\ s_t\ c_u\ s_r$ | 1 |
| 1, 5, 7 | $r_u\ D\ c_u\ s_r$ | 3 |
| | $e_y\ h\ t_h\ s_t\ e^s\ c_a$ | 1 |
| 2, 5, 7 | $r_u\ h\ D\ s_r$ | 1 |
| Total triple crossovers | | 16 |
| 1, 5, 8, 9 | $h\ t_h\ s_t\ c_a$ | 1 |
| Total quadruple crossovers | | 1 |
| Grand total | | 1733 |

TABLE 15

*Translocation e.*

$e_y \quad r_u\ h\ 2\ 3\ t_h\ s_t\ 5\ 6\ c_u\ s_r\ e^s\ c_a$

———————————— ♀ × $e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ ♂.

.. 1 *D* 4 + 7 8 9

| CLASSES OF THE OFFSPRING | NUMBER OBSERVED |
|---|---|
| 0 $D$ | 285 |
| 0 $e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ | 81 |
| Total non-crossovers | 366 |
| 1 $r_u\ D$ | 135 |
| 1 $e_y\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ | 24 |
| 2 $r_u\ h\ D$ | 49 |
| 2 $e_y\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ | 43 |
| 3 $e_y\ D\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ | 1 |
| 3 $r_u\ h$ | 6 |
| 4 $e_y\ D\ s_t\ c_u\ s_r\ e^s\ c_a$ | 2 |
| 4 $r_u\ h\ t_h$ | 2 |
| 5 $e_y\ D\ c_u\ s_r\ e^s\ c_a$ | 6 |
| 5 $r_u\ h\ t_h\ s_t$ | 6 |
| 6 $D\ c_u\ s_r\ e^s\ c_a$ | 20 |
| 6 $e_y\ r_u\ h\ t_h\ s_t$ | 14 |
| 7 $D\ s_r\ e^s\ c_a$ | 117 |
| 7 $e_y\ r_u\ h\ t_h\ s_t\ c_u$ | 27 |
| 8 $D\ e^s\ c_a$ | 85 |
| 8 $e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r$ | 33 |
| 9 $D\ c_a$ | 199 |
| 9 $e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s$ | 67 |
| Total single crossovers | 836 |
| 1, 9 $r_u\ D\ c_a$ | 57 |
| 1, 9 $e_y\ h\ t_h\ s_t\ c_u\ s_r\ e^s$ | 24 |
| 2, 9 $r_u\ h\ D\ c_a$ | 39 |
| 2, 9 $e_y\ t_h\ s_t\ c_u\ s_r\ e^s$ | 7 |
| 3, 9 $r_u\ h\ c_a$ | 1 |
| 3, 9 $e_y\ D\ t_h\ s_t\ c_u\ s_r\ e^s$ | 1 |
| 4, 9 $r_u\ h\ t_h\ c_a$ | 1 |
| 4, 9 $e_y\ D\ s_t\ c_u\ s_r\ e^s$ | |
| 5, 9 $r_u\ h\ t_h\ s_t\ c_a$ | 1 |
| 6, 9 $e_y\ r_u\ h\ t_h\ s_t\ c_a$ | 1 |
| 6, 9 $D\ c_u\ s_r\ e^s$ | 7 |
| | 5 |

| CLASSES OF THE OFFSPRING | NUMBER OBSERVED |
|---|---|
| 7, 9 $D\ s_r\ e^s$ | 26 |
| 7, 9 $e_y\ r_u\ h\ t_h\ s_t\ s_u\ c_a$ | 6 |
| 8, 9 $D\ e^s$ | 5 |
| 8, 9 $e_y\ r_u\ h\ t_h\ s_t\ c_u\ s_r\ c_a$ | 5 |
| 1, 8 $r_u\ D\ e^s\ c_a$ | 17 |
| 2, 8 $r_u\ h\ D\ e^s\ c_a$ | 10 |
| 3, 8 $r_u\ h\ e^s\ c_a$ | 6 |
| 5, 8 $r_u\ h\ t_h\ s_t\ e^s\ c_a$ | 6 |
| 6, 8 $D\ c_u\ s_r$ | 3 |
| 6, 8 $e_y\ r_u\ h\ t_h\ s_t\ e^s\ c_a$ | 1 |
| 1, 7 $r_u\ D\ s_r\ e^s\ c_a$ | 20 |
| 1, 7 $e_y\ h\ t_h\ s_t\ c_u$ | 10 |
| 2, 7 $r_u\ h\ D\ s_r\ e^s\ c_a$ | 11 |
| 2, 7 $e_y\ t_h\ s_t\ c_a$ | 7 |
| 3, 7 $r_u\ h\ s_r\ e^s\ c_a$ | 1 |
| 4, 7 $r_u\ h\ t_h\ s_r\ e^s\ c_a$ | 1 |
| 2, 6 $r_u\ h\ D\ c_u\ s_r\ e^s\ c_a$ | 2 |
| 2, 5 $t_h\ s_t$ | 1 |
| Total double crossovers | 282 |
| 1, 8, 9 $r_u\ D\ e^s$ | 1 |
| 1, 7, 9 $r_u\ D\ s_r\ e^s$ | 1 |
| 1, 6, 9 $e_y\ h\ t_h\ s_t\ c_a$ | 1 |
| 5, 7, 9 $r_u\ h\ t_h\ s_t\ s_r\ e^s$ | 2 |
| 2, 8, 9 $e_y\ t_h\ s_t\ c_u\ s_r\ c_a$ | 1 |
| 1, 2, 9 $e_y\ r_u\ t_h\ s_t\ c_u\ s_r\ e^s$ | 1 |
| 6, 7, 9 $e_y\ r_u\ h\ t_h\ s_t\ s_r\ e^s$ | 1 |
| Total triple-crossovers | 8 |
| 1, 2, 7, 9 $e_y\ r_u\ t_h\ s_t\ c_u\ c_a$ | 1 |
| 1, 2, 7, 9 $h\ D\ s_r\ e^s$ | 3 |
| Total quadruple crossovers | 4 |
| Grand total | 1496 |

TABLE 16

*Crossing over in the normal third chromosome—control experiment.*

$$\frac{e_y \quad r_u \; h \; 2 \; 3 \; t_h \; s_t \; c_u \; s_r \; e^s \; c_a}{+ \quad 1 \quad D \quad 4 \; 5 \; 6 \; 7 \; 8} \; ♀ \times e_y \, r_u \, h \, t_h \, s_t \, c_u \, s_r \, e^s \, c_a ♂.$$

| CLASSES OF THE OFFSPRING | | NUMBER OBSERVED |
|---|---|---|
| 0 | $D$ | 354 |
| | $r_u \; h \; t_h \; s_t \; c_u \; s_r \; e^s \; c_a$ | 165 |
| Total non-crossovers | | 519 |
| 1 | $r_u \; D$ | 144 |
| | $h \; t_h \; s_t \; c_u \; s_r \; e^s \; c_a$ | 56 |
| 2 | $r_u \; h \; D$ | 76 |
| | $t_h \; s_t \; c_u \; s_r \; e^s \; c_a$ | 46 |
| 3 | $r_u \; h$ | 6 |
| | $D \; t_h \; s_t \; c_u \; s_r \; e^s \; c_a$ | 6 |
| 4 | $r_u \; h \; t_h$ | 11 |
| | $D \; s_t \; c_u \; s_r \; e^s \; c_a$ | 2 |
| 5 | $r_u \; h \; t_h \; s_t$ | 44 |
| | $D \; c_u \; s_r \; e^s \; c_a$ | 28 |
| 6 | $r_u \; h \; t_h \; s_t \; c_u$ | 46 |
| | $D \; s_r \; e^s \; c_a$ | 87 |
| 7 | $r_u \; h \; t_h \; s_t \; c_u \; s_r$ | 41 |
| | $D \; e^s \; c_a$ | 57 |
| 8 | $r_u \; h \; t_h \; s_t \; c_u \; s_r \; e^s$ | 77 |
| | $D \; c_a$ | 218 |
| Total single crossovers | | 945 |
| 1, 8 | $r_u \; D \; c_a$ | 110 |
| | $h \; t_h \; s_t \; c_u \; s_r \; e^s$ | 23 |
| 2, 8 | $r_u \; h \; D \; c_a$ | 53 |
| | $t_h \; s_t \; c_u \; s_r \; e^s$ | 23 |
| 3, 8 | $r_u \; h \; c_a$ | 3 |
| | $D \; t_h \; s_t \; c_u \; s_r \; e^s$ | 1 |
| 4, 8 | $D \; s_t \; c_u \; s_r \; e^s$ | 2 |
| 5, 8 | $r_u \; h \; t_h \; s_t \; c_a$ | 27 |
| | $D \; c_u \; s_r \; e^s$ | 7 |
| 6, 8 | $r_u \; h \; t_h \; s_t \; c_u \; c_a$ | 13 |
| | $D \; s_r \; e^s$ | 26 |
| 7, 8 | $D \; e^s$ | 13 |
| | $r_u \; h \; t_h \; st \; c_u \; s_r \; c_a$ | 3 |
| 1, 7 | $r_u \; D \; e^s \; c_a$ | 28 |
| | $h \; t_h \; s_t \; c_u \; s_r$ | 18 |
| 2, 7 | $r_u \; h \; D \; e^s \; c_a$ | 12 |
| | $t_h \; s_t \; c_u \; s_r$ | 9 |
| 3, 7 | $r_u \; h \; e^s \; c_a$ | 2 |
| | $D \; t_h \; s_t \; c_u \; s_r$ | 1 |
| 4, 7 | $D \; s_t \; c_u \; s_r$ | 1 |
| 5, 7 | $D \; c_u \; s_r$ | 1 |
| | $r_u \; h \; t_h \; s_t \; e^s \; c_a$ | 10 |
| 7, 6 | $D \; s_r$ | 1 |
| | $r_u \; h \; t_h \; s_t \; c_u \; e^s \; c_a$ | 6 |
| 1, 6 | $r_u \; D \; s_r \; e^s \; c_a$ | 41 |
| | $h \; t_h \; s_t \; c_u$ | 19 |
| 2, 6 | $r_u \; h \; D \; s_r \; e^s \; c_a$ | 17 |
| | $t_h \; s_t \; c_u$ | 15 |
| 3, 6 | $r_u \; h \; s_r \; e^s \; c_a$ | 3 |
| | $D \; t_h \; s_t \; c_u$ | 1 |
| 5, 6 | $r_u \; h \; t_h \; s_t \; s_r \; e^s \; c_a$ | 11 |
| | $D \; c_u$ | 5 |
| 1, 5 | $r_u \; D \; c_u \; s_r \; e^s \; c_a$ | 9 |
| | $h \; t_h \; s_t$ | 6 |
| 2, 5 | $t_h \; s_t$ | 2 |
| 1, 2 | $h \; D$ | 6 |
| | $r_u \; t_h \; s_t \; c_u \; s_r \; e^s \; c_a$ | 1 |
| Total double crossovers | | 528 |
| 1, 6, 8 | $r_u \; D \; s_r \; e^s$ | 7 |
| | $h \; t_h \; s_t \; c_u \; c_a$ | 1 |
| 2, 6, 8 | $r_u \; h \; D \; s_r \; e^s$ | 4 |
| | $t_h \; s_t \; c_u \; c_a$ | 4 |
| 1, 7, 8 | $h \; t_h \; s_t \; c_u \; s_r \; c_a$ | 1 |
| 2, 7, 8 | $t_h \; s_t \; c_u \; s_r \; c_a$ | 2 |
| 1, 5, 8 | $r_u \; D \; c_u \; s_r \; e^s$ | 3 |
| | $h \; t_h \; s_t \; c_a$ | 3 |
| 2, 5, 8 | $t_h \; s_t \; c_a$ | 2 |
| 5, 6, 8 | $r_u \; h \; t_h \; s_t \; s_r \; e^s$ | 1 |
| 5, 7, 8 | $D \; c_u \; s_r \; c_a$ | 1 |
| 1, 2, 8 | $h \; D \; c_a$ | 1 |
| 2, 5, 7 | $r_u \; h \; D \; c_u \; s_r$ | 2 |
| 1, 5, 6 | $h \; t_h \; s_t \; s_r \; e^s \; c_a$ | 2 |
| 2, 5, 6 | $t_h \; s_t \; s_r \; e^s \; c_a$ | 1 |
| 1, 2, 6 | $h \; D \; s_r \; e^s \; c_a$ | 1 |
| Total triple crossovers | | 36 |
| 1, 4, 6, 8 | $h \; t_h \; s_r \; e^s$ | 1 |
| Total quadruple crossovers | | 1 |
| Grand total | | 2029 |

TABLE 17

$\frac{B_l\ D\ +}{+\ +\ e_y}$ ♂ × $\frac{+\ +\ e_y}{+\ +\ e_y}$. *Summary of counts in the "normal" cultures.*

| | WILD TYPE | $B_l$ | $D$ | $e_y$ | $B_l D$ | $D e_y$ | $B_l e_y$ | $B_l D e_y$ |
|---|---|---|---|---|---|---|---|---|
| Females | 1152 | 1145 | 1163 | 1052 | 1191 | 986 | 1023 | 1016 |
| Males | 1044 | 1038 | 1083 | 1015 | 1133 | 911 | 1117 | 975 |
| Total | 2196 | 2183 | 2246 | 2067 | 2324 | 1897 | 2140 | 1991 |

Grand total 17044

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